THE BLOOD PLASMA
IN HEALTH AND DISEASE

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EDITOR'S PREFACE

THE art of medicine is old and time-honoured, but the science of medicine is relatively new, and may be considered to have begun with Harvey's discovery of the circulation of the blood. Meantime new sciences have been born, have so multiplied and have become so specialised that it is no longer possible for an individual to keep abreast with the recent developments. General text-books necessarily lag behind progress, and too often original papers contain so much technical detail that they can be fully appreciated only by specialists themselves. This is very true of medical science.

There are, however, in every country, a few individuals who are interested in the more scientific aspect of medical and surgical advance, who sift accumulated fact in the more basal sciences, in some instances with a view to furthering this advance, and in others in the disinterested pursuit of knowledge, although inevitably they must confine their efforts to limited fields.

The object of this series of monographs is to provide a medium by which the knowledge they have collected may become generally available. It is intended that so far as possible the monographs should be of value not only to the research worker, but, by the avoidance of undue technical detail in the applied chapters, that they should also be of practical use to the clinician.

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AUTHOR'S PREFACE

Blood plasma has been investigated from the standpoints of biochemistry, physiology and pathology. Each of these branches of research has revealed important truths, but the differences of outlook among workers have often led to the treatment of this subject in compartments. An attempt will be made, within the limits of a small volume, to correlate certain facts derived from each of these sources of information, and to point out some of their practical applications.

Pathologists have often described the formation of blood clots in living animals as "necrosis," thus implying that circulating plasma is a living fluid. Even if this be true, changes in plasma should ultimately be expressible in physico-chemical terms. For this reason, I shall use the interpretations of physics and chemistry as far as I am able. General conclusions will, however, be suggested only when the biological and physico-chemical phenomena point in the same direction.

Most works on haematology are almost wholly devoted to the histology of blood corpuscles and of the haematopoietic system. Except in the study of haemophilia and of thrombosis, blood plasma is either neglected or treated merely as a vehicle for the conveyance of corpuscles. A different outlook will be selected. Evidence will be collated which suggests that the plasma is of fundamental importance in the life of the whole body, and it will be shown that morbid conditions appear when the stability of plasma is altered.

Modern knowledge of the plasma is largely derived from the investigation of the precipitability and coagulability of that fluid. Attention will, therefore, be given to these inquiries before discussing changes in the behaviour of the blood *in vivo* and their significance in certain pathological processes.

Except in one instance, the nomenclature in common use in this country will be employed, but the different shades of meaning implied in the terminologies of foreign writers will be explained.

It is a very pleasant duty to record my most cordial thanks to Prof. Howell of Baltimore, to Prof. Nolf of Liége, to Dr. Hekma of Hoorn, and to Dr. C. A. Mills of Cincinnati, for the summaries of their theories of blood coagulation, which appear in Chapter X. I am indebted to Prof. Bulloch, of London, for reading the discussion of the genetics of hæmophilia and to Dr. F. R. Curtis for the onerous work of reading the proofs.

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BLOOD PLASMA IN HEALTH AND DISEASE

CHAPTER I

Introductory and Historical

An appreciation of the constitution and behaviour of blood plasma opens up many highways of inquiry both of practical and theoretical importance. On the practical side we may anticipate improved methods of arresting obstinate and inaccessible hæmorrhages, the safeguarding of intravenous therapy, and a more scientific treatment of several obscure conditions, such as those found in thrombosis, in hæmophilia and in many other hæmorrhagic disorders. On the theoretical side light should be thrown on the behaviour of colloids in the living organism—a fundamental problem in biochemistry—on the functions and "life cycle" of plasma protein, on the relations of humoral and cellular reactions, on the changes produced in plasma by medicaments, on the phenomena of anaphylactic shock, as well as those of susceptibility, of tolerance and of immunity. Also on the problems of genetics, since hæmophilia and allied disorders are transmitted from parent to offspring, whilst the existence of homologous bloods often depends on family relationship.

Apart from metaphysical speculations, like those of Harvey (1653), who conceived the blood as the very fountain of life—*primum movens, ultimum moriens*,—the early physiologists were interested only in the fact that blood is found clotted when shed, but they offered no explanation of this occurrence.

The first step towards modern knowledge was taken by Malphigi (1666), who found that a mass of white fibres

remains after the washing of blood clots. Borelli (1681) foreshadowed, but did not demonstrate, the discovery of Hewson (1770) that, in clotting, a solid separates from the *liquor sanguinis*. Ruysch (1707) had, however, stated that the elastic material obtained by whipping blood was similar to that found after washing clots. It was named fibrin by Chaptal (1795) and became generally known through the work of Fourcroy (1804). Fibrin was said to exist in the plasma and clotting to be due to spontaneous solidification. The first of these opinions partly anticipated the recent suggestions of Hekma (1916–1927), the second was contested by Buchanan (1835, 1845), who found that the addition either of leucocytes or of serum to certain serous fluids produces a coagulum of fibrin. The latter investigator compared blood coagulation to the action of rennin in the clotting of milk and thought it was brought about by the colourless corpuscles.

The earliest application of analytical methods was the use of the precipitating action of neutral salts in the separation of protein aggregates (Panum, 1852; Bernard, 1853; Virchow, 1854). Using this method, Denis (1859) saturated with NaCl a plasma kept fluid by the addition of one-seventh of its volume of Na_2SO_4. A gelatinous mass was precipitated, named "plasmine," which dissolved in water and subsequently coagulated at room temperature, giving fibrin and a protein left in solution. From this experiment, Denis concluded that the precursor of fibrin suffers cleavage in the act of clotting, an opinion supported by subsequent investigation. This work almost passed into oblivion, owing to the importance attached to the methods of purifying plasma precipitates devised by later workers. It seems, however, that the very lack of these methods permitted Denis to handle material more akin to the protein of freshly shed plasma than is possible when more refined processes are used.

The facts described by Buchanan were re-discovered by Alexander Schmidt (1861, 1862). He obtained from plasma and from serum respectively, after dilution with fifteen to twenty volumes of water and the passage of a stream of

CO_2, two protein precipitates which he named "fibrinogen" and "paraglobulin." In his earlier works the clotting of blood was regarded as due to the union of these materials, the latter being described as the active substance. Later, Schmidt (1892) obtained from both blood clots and serum, by prolonged extraction with alcohol, a residue which was soluble in water and rapidly clotted liquids containing fibrinogen and paraglobulin (the serum globulin of later writers). This coagulant was provisionally named "fibrin ferment" or "thrombin." It was said to produce clotting by uniting fibrinogen and paraglobulin. By receiving blood directly from an artery into alcohol and using the same process that yielded thrombin from serum, he demonstrated the absence of that coagulant in circulating blood. The presence of some antecedent of thrombin in blood became evident, and it was named "prothrombin." It was thus shown that important changes in blood precede clotting.

In many respects, Hammarsten (1877–1879) may be regarded as the originator of the modern outlook, with one important reservation. Unlike many who followed him, he did not postulate the existence in plasma of substances that can be obtained only by the drastic disintegration of colloidal complexes. By treating plasma with various concentrations of NaCl, he showed that less of that salt is required for the precipitation of fibrinogen than suffices for the salting-out of serum globulin. He also demonstrated that serum globulin does not participate in the formation of clots, and thus concluded that coagulation arises from the action of thrombin on a single constituent of plasma—to wit, fibrinogen.

Two widely different modes of research are now in use. Both were employed by Hewson (1770, 1772), who performed experiments on living animals, and by observations on salted bloods laid the foundation of biochemical methods. *In vivo* the plasma cannot be either isolated or analysed, and the reactions of living cells and tissues may be involved. For these reasons, precedence has been given to chemical investigations and contemporary knowledge rests largely on

reactions in test tubes. Results so obtained do not afford, however, any certitude that the materials examined are in a similar condition to those of circulating blood, since the outstanding feature of shed blood is a tendency to immediate change, and this is increased whenever blood is in contact with a surface which it wets.

Many attempts have been made to avoid this instability by investigating plasma kept fluid by the addition either of an excess of water or of specific anticoagulants. Dilution with sufficient water to suppress clotting alters the plasma, since evaporation to its original volume does not restore the capacity to clot spontaneously. Later, it will be shown that the effects produced by anticoagulants on shed blood often differ from those occurring in circulating blood. Experiments on plasma kept fluid by these methods have, therefore, a limited meaning, and several current generalisations which are derived from this mode of research do not satisfy the criterion mentioned in the preface.

Except in a few experiments on oxalated plasmas deprived of blood platelets by passage through a Berkefeld filter, and in those on the plasma of the domestic fowl from which all formed elements have been removed in an intact condition, all the plasmas used in biochemical research have been contaminated with the *débris* of blood platelets—with material which rapidly alters the stability of plasma. The difficulty of interpreting results unfortunately increases whenever attempts are made to purify plasma protein by precipitation and re-solution, as these processes alter labile colloids. So far, synthetic methods have been only tentatively employed, and investigators must await the production of a fluid exhibiting all the qualities of blood plasma by the reunion of its components. Only then can they be sure of the possession of these bodies.

Perhaps the most distinctive feature of the current literature is the multiplicity of hypotheses. Some of these speculations are almost irreconcilable one with another, others outrun the bounds of fact. Almost all assume that blood plasma consists of a number of distinct substances that interact both *in vivo* and *in vitro*. The behaviour of

undisturbed plasma and the possibility of its disintegration in the inception of clotting have attracted comparatively little attention. For these reasons, I shall first consider the classification of fractions of plasma protein and their probable relationship in undisturbed blood.

CHAPTER II

The Protein of Blood Plasma in Fractions and as a Whole

Classification by precipitability—The condition of the fractions of protein obtained by salting-out and that of the protein of plasma—Other classifications—Variations in the stability of blood plasma and possibly in the stability of the blood in carcinoma—Embryonic development—The supposed transformation of one protein fraction into another—The functions of plasma—The hereditary transmission of certain conditions of plasma.

THE work of Hammarsten led to the classing of the proteins of plasma and of serum by their behaviour in the presence of neutral salts. This classification is based on the finding that fractions of protein possessing distinctive properties can be precipitated by definite concentrations of salts, those commonly used being NaCl, $(NH_4)_2SO_4$ and $MgSO_4$. The following table, modified from that compiled by Davide (1925), gives an approximate idea of the limits of precipitability by these salts.

TABLE I.—*Illustrating the Limits of Precipitability of the Principal Fractions of Protein obtainable from Blood Plasma.*

Precipitants.	Fibrinogen	Euglobulin.	Pseudo-globulin.	Albumin.
NaCl. .	Precipitated at nearly half saturation.	Precipitated on saturation.	—	—
$(NH_4)_2SO_4$.	Precipitated on the addition of 15–27 per cent. of a saturated solution.	Precipitated on the addition of 28–38 per cent. of a saturated solution.	Precipitated on the addition of 36–44 per cent. of a saturated solution.	Precipitated on saturation.
$MgSO_4$.	Precipitated at nearly half saturation.	Precipitated on saturation.	Precipitated on saturation.	—

This classification neglects prothrombin and fibrin-globulin, as well as the somewhat hypothetical seromucoid.

Other observers record rather different figures. For example, Porgès and Spiro (1902) state that the percentages of solutions of saturated $(NH_4)_2SO_4$ necessary for precipitations are as follows: Fibrinogen 30 per cent., euglobulin 30–36 per cent., pseudo-globulin 36–50 per cent., albumin 50–100 per cent. They also distinguish two fractions of pseudo-globulin, one precipitated by 36–44 per cent., the other by 44–50 per cent. A recent application of salting-out has led to the expression of the limits of the precipitability of the various protein fractions in terms of the fractional volume-molar concentrations of different salts. After fibrinogen has been precipitated, the additional amount of salt required for the precipitation of another fraction is constant for any salt. The increment of salt may, however, be different for different salts (Howe, 1921, 1922, 1925).

The Condition of the Fractions of Protein obtained by Salting-out and that of the Protein of Plasma

The fractions of protein obtained by salting-out dissolve on appropriate dilution with water. Convenient methods are thus provided for their separation and partial purification, but the precise composition of these precipitates and solutions remains unknown. The work of Sørensen (1917) on the salt-precipitation of albumin shows that the precipitates are of great complexity. There are present dissociated and non-dissociated albumin and the precipitant salt; also combinations of these bodies, as well as water, together with hydrogen and hydroxyl ions. According to the same investigator, the products of the salting-out of globulin are even more complex, since it is not possible to obtain either pure euglobulin or pure pseudo-globulin, but only mixtures of these bodies with associated salts and water.

The only sharply defined difference between euglobulin and pseudo-globulin is their behaviour in water; the former is insoluble, the latter is soluble. Recent work suggests, however, that this distinction no longer holds good. The prolonged electro-dialysis of either normal or anti-toxic serum precipitates all the protein that is usually called globulin. Pseudo-globulin actually requires a small amount of salt to hold it in solution, but the quantity is too small for removal by ordinary dialysis. (Pauli, 1924; Adolf,

1924). These observations are consistent with an earlier conclusion that the products of the salt precipitation of serum globulin, although fairly well defined, are fractions of a larger complex and not chemical units (Herzfeld and Klinger, 1917, 1920. A similar view is taken by Reymann, 1924).

There is, however, some evidence which suggests that euglobulin and pseudo-globulin may differ chemically. The dialysis of pseudo-globulin yields a precipitate containing phosphorus and a water-soluble globulin, which is almost free from phosphorus. The addition of lecithin to the water-soluble material gives, on renewed dialysis, a precipitate containing phosphorus. This implies that euglobulin may be a compound of water-soluble globulin and a lipoid containing phosphorus (Chick, 1914). The prolonged dialysis of prothrombin (which possesses the solubilities of globulin) also gives a water-soluble fraction free from phosphorus and a precipitate containing phosphorus (Cekada, 1926). In both groups of experiments, contamination with phosphatides derived from disintegrated blood platelets was not eliminated. The precipitation of euglobulin from serum by appropriate acidification has been recently explained as due to the formation of a protein-lipoid complex. It is stated that the acid alters the protein and renders this union possible (Wadsworth and collaborators, 1927).

Broadly described, blood serum is plasma divested of fibrinogen. The protein of serum is electrically inactive—it will not move in an electric field. It can, however, be made to move by appropriate treatment, and the character of the movement is such as could hardly be exhibited by a mixture of colloids. In short, it is probable that there is only one protein in serum (Hardy, 1903).

In a discussion of the solubilities of globulins, Sørensen (1925) assigns different formulæ to euglobulin and pseudo-globulin, and thus implies that these bodies are different substances. This is dissonant with the conclusion of Hardy and is opposed by the previously cited observations of Pauli (1924), which are consistent with Hardy's inference in so far as it applies to globulin. In a later paper, Sørensen (1926) more fully explains his position. He suggests that the more complex proteins consist, in the main, of larger or smaller polypeptide complexes, loosely knit together by secondary bindings, or by mutual salt formation taking

place, without any real chemical union between the complexes. Such complexes act in solution as a co-ordinated whole, as is indicated by the slight osmotic pressure they exert. The phenomena of the fractional salt-precipitation of globulin are explained not on the assumption that mixtures of globulin are present, but by suggesting that the globulins are loosely bound one with another into a larger whole. In representing globulins, in the earlier paper, by the formula E_pP_q, it was intended to explain that in the different globulins obtained by salting-out different numbers of euglobulin complexes (E) and pseudo-globulin complexes (P) are present. It follows, if these conclusions represent the truth, that serum globulin is not a simple mixture of different proteins in solution, but is built up of complexes which are loosely held together without the intervention of real chemical reactions.

There are difficulties in the complete acceptance of Hardy's views, such as the chemical and immunological differences between albumin and globulin, described later in this chapter. It seems probable, however, that in fresh serum albumin is attached to globulin by forces similar to those which hold fractions of globulin together. In this mental picture the chemical and immunological differences of albumin and globulin appear of secondary importance. Globulin and albumin are seen as a complex, which behaves like a single substance so long as it is not dissociated by the forces involved in chemical, physical or immunological reactions.

Many important questions remain unanswered, especially the extent of the alteration of protein complexes by salting-out and by electro-dialysis. Proteins reveal apparent dissociation constants at neutral reactions that known amino-acids do not possess, and it is probable that the dissociation of proteins depends upon the dissociation of the amino-acids of which they are composed (Cohn, 1925).

Several observations suggest that the fibrinogen known in the laboratory is a group of proteins more or less firmly bound together. By cataphoresis it can be separated into fractions which react differently with thrombin (Howell,

1916). It is firmly united with prothrombin (Mellanby, 1909, 1). On heating to 56°–60° it yields coagulated protein and uncoagulated fibrin-globulin (Huiscamp, 1905; Iscovesco, 1906).

The suggestion of Mellanby (1909, 1) that prior to clotting, the globulins of serum are united with fibrinogen is consistent with conclusions reached by widely different modes of research. By estimations of four variables in a given blood, Henderson (1921) found that when values are assigned to any two of these variables the values of the others are determined and the condition of equilibrium is unequivocally defined. Hence, he concluded that blood is a single physico-chemical system. From the general behaviour and chemical structure of proteins, Ruppel and his collaborators (1922–1923) infer that the protein fractions of plasma do not exist in a free condition. Furthermore, in the clotting of shed blood one portion of protein suffers change and separates from solution, whilst another portion remains in solution. It seems clear that this is primarily due to the lysis of a protein complex, since a considerable number of substances that restrain the inauguration of blood clotting, but have no influence on the later phases of that change, also possess the capacity of suppressing the lysis of other colloidal complexes, *e.g.*, those of erythrocytes (Pickering and Taylor, 1924).

A reunion and probably a fresh grouping of the material broken up by clotting occurs rapidly in shed mammalian blood, since thrombin is found both bound to fibrin and "inactivated" in serum, whilst the unused prothrombin and the *débris* of blood platelets are soon so firmly united to serum protein that disruptive agencies, such as adsorbents or chloroform, are required for their release.

In salting-out the cleaving is more marked and the fractions of protein obtained may be regarded as products of such action. Biochemists should, however, await further advances in physics and chemistry before speaking definitely on the mode of union of fractions of plasma protein. The work of Bayliss (1921, 1924) points to physical unions playing an important part, and the use of the term "adsorp-

tion," in contradistinction to true chemical action, is at least convenient in the present state of knowledge. The completely different teaching of J. Loeb (1924 ·2) should, however, be remembered. He states that proteins combine stoichiometrically with acids and alkalis and that there are no differences between the chemistry of proteins and that of crystalloids. The application of the stoichiometrical law and of Donnan's theory of membrane equilibria is said to suffice for the explanation of colloidal behaviour.

Reviewing the facts and inferences as a whole, it seems probable that undisturbed plasma is a single complex and not a mixture of disunited substances. If this suggestion is ultimately established, blood plasma will be regarded as a co-ordinated whole, which acts as a unit so long as its components are not dissociated by extraneous forces. This conclusion satisfies the criterion mentioned in the preface. It is consistent with both the physico-chemical reactions *in vitro* and the biological phenomena. Distinctive reactions that are readily obtainable with fractions of plasma *in vitro*, such as the formation of thrombin and the clotting of fibrinogen by thrombin, do not occur *in vivo* unless the equlibrium of the plasma is disturbed.

A bolder conclusion was reached by Wooldridge (1886). He revived the conception of Harvey (1653) that the blood is a living liquid. He maintained that blood plasma is protoplasm and that clotting is the last act of living blood. This fascinating speculation should not be ignored, but it can hardly assist progress in the present state of knowledge. If, however, the "intuition" of Wooldridge represents truth, then the meaning of the changes that transform living bodies into dead material lies hidden in the problem of blood coagulation.

Other Classifications

An alternative classification is provided by the differences in thermostability exhibited by fractions of plasma protein. The exhaustive investigation by Halliburton (1884–5, 1886, 1891) of the heat coagulation of plasma and of serum, shows a remarkable concordance of results. In the plasmas of

both higher and lower vertebrates, three fractions of serum albumin are found, coagulating respectively at 73°, 78° and 85° C. In lower vertebrates, except in the eel, only one fraction is evident, coagulating at 73°–74° C. Broadly speaking, classification by heat coagulation corresponds with that based on salting-out, but the incidence of heat coagulation is much more sharply defined than is precipitation by $(NH_4)_2SO_4$. The quantities of protein obtained by these methods show a close but not complete correspondence.

The chief interest of heat coagulation is the evidence it affords of the existence of different fractions of albumin in blood plasma. Similar evidence is provided by salt-precipitation, definite limits of precipitability being claimed (Oppenheimer, 1903), by the immunological specificity of albumin fractions (Doerr and Berger, 1922 1·2) and by certain changes in albumin fractions during alterations in environmental conditions (Howe and Sanderson, 1924). But such evidence does not establish the existence of these fractions in a free condition in undisturbed plasma.

Variations in the Stability of Plasma and possibly in the Stability of the Blood in Carcinoma

The blood plasmas of animals of different species exhibit varying degrees of stability. Mammalian plasmas clot rapidly when shed, those of birds and fishes may remain fluid for long periods (Delezenne, 1897; Nolf, 1906, 1909), but can be clotted by prolonged shaking and by the addition either of chemically inert particles or of tissue extracts. Decreased coagulability of the blood will be described in certain hæmorrhagic conditions. It will be shown that this may occur when the fractions of plasma which participate in blood clotting are neither deficient in quantity nor altered in quality and when there is no evidence of an excess of anticoagulant in the blood. It seems probable that variations occur in the manner in which the proteins of plasma are held together.

Well-marked suppressions of blood clotting occur in pneumonia (Dochez, 1912), but this may be due to the liberation of anticoagulants by the necrosis of the endo-

thelium of the lungs. The blood of persons who are critically ill may exhibit changes in the speed of clotting. This occurs during widely different disorders (Addis, 1910; Lee and White, 1913). It is not sufficiently defined for use in diagnosis. Similar changes were found by Van Allen (1927) during the height and terminal stages of various disorders in rabbits, including epithelioma. But they were not apparent in gradually developing maladies, even when the emaciation was profound. Decreases in the rate of contraction of blood clots during morbid states, particularly in malignant tumours, will be described later and may be of diagnostic value if similar changes occur in human blood.

Recent investigations suggest that the stability of blood plasma is altered during the development of carcinoma. Wegierko (1926) states that in the majority of sixty cases which he examined the blood is less thermostable than that of normal persons. This change is said to be correlated with the progress of the malignant growth. In the early development of carcinoma, the capacity of the plasma to flocculate at 52°–54° C. is small. When the growth is well marked flocculation is readily obtained, but the capacity of the plasma to flocculate at these temperatures almost disappears during the final stages of the neoplasm if cachexia is extreme. Alterations in the thermostability of plasma are also described in other morbid states, such as diphtheritic pneumonia, acute articular rheumatism, and when certain changes have occurred in the renal endothelium.

Bock and Rausche (1926) claim that a smaller quantity of $MgSO_4$ is required for the prevention of the clotting of the blood of persons suffering from cancer than suffices when the blood either of normal persons or of those suffering from other diseases is used. They state that in sixteen out of eighteen cases of cancer, which were verified by operation, three to five drops of a 2 per cent. solution of $MgSO_4$ were sufficient for the prevention of the clotting of 1 c.c. of blood and, that seven to eleven drops are necessary for the suppression of the clotting of an equal amount of the blood of normal persons. In thirty-five bloods obtained from persons suffering from other diseases, three to six drops were required. These experiments should be repeated on a

larger scale, on account of their possible importance in the diagnosis of cancer. The results obtained should be compared with the precipitability of the plasmas by neutral salts after dilution, with the amount of fibrinogen present, and with the ultramicroscopic changes occurring during clotting. By such methods it should be possible to ascertain if there is any marked change in the stability of plasma in carcinoma.

Embryonic Development

Although much attention has been given to the embryology of blood corpuscles, that of plasma has almost escaped notice. This neglect is, indeed, surprising, since current speculations on the anabolism of protein and on the supposed transformation of one protein into another are based either on changes in abnormal states or on reactions *in vitro*, without checking the conclusions reached by comparison with the data disclosed by embryonic development.

A few facts are, however, known. In the second-day chick certain mesodermal cells, called "angioblasts," differentiate and produce endothelium, erythrocytes and plasma. It is thus probable that blood plasma arises in the embryo from the destruction of cells (Sabin, 1920). The blood of chick embryos does not clot prior to the twelfth day of incubation (Boll, 1870). The lack of coagulability is due to the absence of fibrinogen. On the eleventh day the protein of plasma possesses the solubility of albumin and on heating coagulates in fractions at 72°–75° and 80° C. respectively. Globulin is absent. On the thirteenth or fourteenth day fibrinogen and prothrombin develop simultaneously. In some thirteenth-day embryos fibrinogen is not present, and in these embryos other globulin is also absent. On the fourteenth day the shed blood may clot without leaving any appreciable amount of globulin in the serum. In older embryos both globulin and albumin are easily separated from serum (Pickering and Gladstone, 1925, 2). That embryonic development proceeds in the same order as evolutionary development seems established. It follows that albumin is nearer the primitive type of protein than globulin. The conclusion of Sørensen (1923) that molecules (or aggregates) of globulin are larger and therefore

more complex than those of albumin points in the same direction—that albumin is less differentiated than globulin. It seems that there is an evolution of colloidal complexity as well as an evolution of anatomical structures.

In embryo chicks the capacity of shed blood plasma to agglutinate its own thrombocytes is absent prior to the tenth day of incubation, but is well marked in embryos aged fourteen days. The power of agglutinating red corpuscles is absent before the eleventh day, but the plasma of some twelfth-day embryos may slowly and partially agglutinate the erythrocytes of the rabbit and guinea-pig. The appearance of the capacity of the plasma to clot does not coincide with an increase in the power to agglutinate. Complement could not be detected before the thirteenth day, and on that day only in those plasmas which had developed fibrinogen and prothrombin. During later stages of development complement is present in gradually increasing amounts in both plasma and serum. Material lytic to the erythrocytes of the rabbit and guinea-pig could not be found prior to the thirteenth day, but from that day onwards it is present in variable amounts in both plasma and serum.

These observations suggest that the capacity to agglutinate corpuscles is a property of the albumin of plasma, and that albumin must reach a certain degree of complexity to acquire this quality. It is also indicated that complement is associated with globulin, and there is less conclusive evidence that hæmolytic power is due to similar material (Pickering and Taylor, 1927).

These conclusions only broadly correspond with those reached from the study of the reactions of the serums of adult mammals. Priestly (1915) suggests that the agglutination of heterologous erythrocytes is caused by serum protein, probably by globulin. Maltaner and Johnston (1921) state that the degree of agglutination or hæmolysis obtained with a "heat-sensitive" serum depends, in part at least, on the quantity of fibrinogen present, and that agglutination is due to the formation of filaments of fibrin. The association of complement with a complex containing globulin is, however, generally accepted, and a relationship has been recently claimed between the stability of the blood, as indicated by its coagulability, and the amount of "complement activity" in serum (Fuchs and v. Falkenhausen, 1927).

The Supposed Transformation of one Fraction of Plasma Protein into another

A decision on this question is of considerable importance in relation to the metabolism of protein and in the investiga-

tion of several obscure changes in the coagulability of the blood, particularly those in anaphylactic and "peptone" shock. If one plasma protein is transformed into another an explanation of change in the stability of the plasma is offered. If, however, such transformations do not occur, we must search for alterations in physical condition, for possible dissociation and re-grouping of plasma complexes, and for changes in hydrion concentration.

Many investigators claim that the increase of fibrinogen or of serum globulin found in certain morbid states and during immunisation is at the expense of albumin, since the ratio $\frac{\text{albumin}}{\text{globulin}}$ is lower in disease and in immunisation than in healthy and non-immunised states (Hoffman, 1882–1883; Hiss and Atkinson, 1900–1901; Joachim, 1902–1903; Ledingham, 1907; Gibson and Banzhaf, 1920). An increase of serum globulin is not, however, invariably accompanied by a decrease of albumin, for in immunisation against tetanus both the globulin and albumin increase, whilst in that against plague a large increase of globulin is accompanied by a slight increase of albumin (Sordelli and Mazococco, 1925). The facts, though suggestive of protein transformations *in vivo*, do not provide conclusive evidence of such changes. The chemical evidence is also indecisive, whilst the serological evidence unequivocally opposes the occurrence of such changes.

Moll (1904) claims the production of *ein Kunstliches Globulin*, by the incubation of a slightly alkaline solution of albumin for an hour at 56°–60° C. The incubated material is precipitated by half saturation with $(NH_4)_2SO_4$, and the precipitate contains a smaller amount of sulphur than albumin. Two of the chief characteristics of globulin are thus present. Similar results were obtained by Kammerer and Aubry (1913), who did not, however, claim the conversion of globulin into albumin, but merely state that a change in precipitability occurs.

More recently, Ruppel and others (1922) state that the incubation of a strongly alkaline solution of albumin at 37° C. for three days yields material possessing all the properties of euglobulin. In a review of this work, Davide (1925) points out that the hydrion concentration of these solutions was not controlled. He also emphasises the differences of natural and artificial globulins.

Natural globulin contains glycocoll and phosphorus, bodies that are absent in albumin. The latter material contains only a minute amount of carbohydrates, whilst the percentage of sulphur is approximately double that of globulin. It has been shown by a number of workers that artificial globulin has a low percentage of carbohydrates and a percentage of sulphur closer to that of albumin than globulin. Moreover, the amino-index (*i e*, the total nitrogen divided by the formol nitrogen) is the same for albumin and artificial globulin, but is twice as high as that of serum globulin (Gibson, 1912; Obermeyer and Willheim, 1912; Bywaters and Tasker, 1913). A change from albumin to globulin in the absence of added alkali is, however, said to occur, the evidence for transformation being based on alterations in refractility (Gutzeit, 1924). But the burden of proof that changes in physical characteristics are not merely examples of "denaturation" rests on those who claim the conversion of one protein into another.

On the serological side, it is established that the different fractions obtainable from serum protein exhibit distinct specificity (Dale and Hartley, 1916; Doerr and Berger, 1922). Further, Fanconi (1923) not only found physical differences between artificial and natural globulins, but also showed immunological differences between the artificial globulins prepared by different methods. Moll's material does not produce shock in guinea-pigs sensitised with natural serum globulin, whilst it sensitises animals more effectively for albumin than for natural globulin. Another "artificial globulin," prepared by a modification of Ruppel's method, is completely ineffective in these respects.

Attempts have been made to convert albumin into globulin by irradiation with ultra-violet light. When salt-free albumin is exposed, at certain hydrion concentrations, to radiations of wave-lengths less than 320$\mu\mu$, a product is obtained which is precipitated, like globulin, either by half saturation with NaCl, or by dialysis (Clark, 1922, 1925). It has, however, been pointed out that irradiation does not change the optical declination of albumin, which should happen if any structural change had occurred in the protein molecules (Davide, 1925). All the evidence thus points to artificial modifications in physical condition rather than to chemical changes.

Despite the facts just narrated, a transmutation of protein complexes in the limited sense implied by Herzfeld and Klinger (1917, 1919, 1920) may possibly occur. According to these investigators, the protein fractions separated by salting-out are an inter-related series of particles of the same substance possessing different degrees of dispersion.

The least dispersed fraction (fibrinogen) is said to be derived from the *débris* of cells and to be converted by gradually increasing dispersion, first into globulin and then into albumin. It is suggested that stabilisation is effected by absorption of lower products of disintegration, such as amino-acids and polypeptides, the smaller and more soluble particles carrying the greater amount of material. These views are partially accepted by Kapsenberg (1925) in so far as there may be a difference between albumin and globulin. They are also tentatively adopted by Reymann (1922), with the important difference that the development is believed to proceed from albumin to globulin. The evidence of embryonic development shows that albumin appears in plasma long before globulin. It implies that in the living organism albumin does not arise from globulin. We have also seen that fibrinogen appears suddenly at a comparatively late stage of development. An autogenic origin is indicated, not formation from either pseudo-globulin or albumin.

The Functions of Blood Plasma

If blood plasma were a fluid elaborated in a laboratory, we should say it fulfilled all the purposes for which it was designed. It provides a medium for the circulation of oxygen-bearing and other corpuscles, also for the transport of carbon dioxide. It is an excellent buffer solution, and by its osmotic pressure, viscosity and concentration of electrolytes is adapted to its surroundings. By the remarkable property of clotting when blood is shed its loss from wounds is minimised.

Other functions of blood plasma are less obvious but are equally real. The stabilising influence of blood serum on body cells seems established. It has been shown that cell injury and "shock" follow the replacement of a large amount of plasma by a suspension of erythrocytes in Locke's solution, and that if dialysed serum is substituted for Locke's fluid, "shock" does not occur (Hurwitz and Whipple, 1918–1919; Whipple and others, 1920). The transfusion of isotonic gum acacia is equally efficacious in the prevention

of wound "shock" (Bayliss, 1916–1920). It thus appears that the protective influence against cell injury depends upon colloidal behaviour, mainly in relation to permeability, and not upon the chemical properties of protein. That serum protects against several types of hæmolysis is also established (Moore and others, 1909; Sutherland and Mitra, 1916; Barker, 1922; Ponder, 1921, 1922, 1923). Similar action is exhibited by various colloids, such as gum acacia, gelatin and egg-white, also to a lesser degree by viscous solutions of sugar. In protection against hæmolysis, particularly against that produced by natural hæmolysins, more is implied than the maintenance of appropriate osmotic conditions, since in strictly isotonic fluids different colloids have different protective values and the optimum hydrion concentration for protection is different for different colloids. Moreover, the removal of calcium ions from plasma by oxalation restrains the hæmolysis produced by heterologous sera, by specific hæmolysins and by cobra venom, whilst hæmolytic action is restored by replacing the calcium ions (Pickering and Taylor, 1923, 1924). It seems probable that restriction of the movements of ions is an important factor in the protection afforded by colloidal complexes against lytic action.

Plasma exhibits greater stabilising power than serum protein, but this power is either reduced or lost when clotting commences. Nolf (1922) found that dog's blood containing an auto-hæmolysin (*i.e.*, material lytic to the dog's own erythrocytes) does not hæmolyse when shed so long as fluidity is preserved, also that hæmolysis commences at the same time as clotting. Furthermore, lethal intravascular injections of cobra venom into the frog do not hæmolyse the blood whilst it remains in the blood vessels, but when the blood is shed into isotonic saline (which is non-hæmolytic to frog's erythrocytes) lysis appears contemporaneously with clotting (Pickering and Taylor, 1924). Material protective against the lysis of colloidal complexes therefore exists in unshed blood plasma, but it loses its protective power in the act of clotting. That the same factors maintain the integrity of both plasma and erythrocytes is not surprising

if we accept the conclusion that unshed blood is a single physico-chemical system. In such a system influences that stabilise a part would stabilise the whole.

Blood platelets remain intact in circulating blood and rapidly disintegrate when the condition of the plasma is altered by contact with any surface which it wets. The stabilisation of platelets by plasma is of considerable importance. By removing bacteria from the blood-stream, platelets provide a defence against infection (Govaerts, 1921; Delcourt-Bernard, 1922; Roskam, 1927). Moreover, their removal from the circulation favours hæmorrhagic states. The loss of the stabilising power when plasma is shed is of equal importance, since the products of the lysis of platelets participate in the inception of blood clotting and so supply protection against hæmorrhage.

An equilibrating influence of blood plasma in the distribution of water between erythrocytes, connective tissues and cells is suggested by Schade (1923, 1924, 1, 2). The same writer is convinced that renal diuresis is due to an increased attraction of body colloids for water, in which the colloids of plasma participate rather than those of solid tissues. The importance of plasma protein in water-regulation is, however, questioned (Oehme, 1924; Handovsky, 1924).

Nevertheless, the work of Starling (1899) shows the bearing of the colloidal concentration of the plasma on the process of renal filtration and suggests that dilution of the plasma is a cause of renal diuresis. He considers that the glomerular filtration continues as long as the pressure in the capillaries is sufficient to overcome the osmotic resistance of the colloids. The discussion of the old dispute whether the pressure or velocity of the blood flow is the important factor is beyond the scope of this work. It has been ably dealt with in the recent monograph of Cushny (1926), who reaches the conclusion that both factors are essential.

An early conception of the chief function of blood plasma is that it transfers nitrogenous nutriment from the intestines to the body generally, or, as Michael Foster (1888) puts it—tissue by the help of lymph lives on blood. Proof of this conclusion, probable though it seems, is still wanting.

In a recent review of this question, Howe (1925) points out that this hypothesis involves either the synthesis of protein from digestion products in the liver, or else direct absorption of food protein. Convincing evidence of the occurrence of the former process is lacking, whilst the latter suggestion is still disputed. A number of the earlier observers stated that protein passes directly from isolated loops of intestine into the circulation. The principal objection to these experiments is that the possibility of the action of digestive enzymes is not excluded (Fischer, 1921). Mills and his collaborators (1923) state, however, that the protein of lung extract passes rapidly from the intestines into the blood-stream and can be recovered unchanged in the urine. If foreign protein commonly passes unchanged into the blood-stream, it is difficult to understand why sensitisation and anaphylactic shock do not frequently occur, unless anti-sensitisation is a common occurrence.

A unique speculation on the mechanism of nutrition is advocated by Nolf (1908). He suggests that blood clotting occurs normally in circulating blood, but is limited to the deposit of films of fibrin on the vascular endothelium. The fibrin is thought to be converted into nutrient material by a fibrinolytic enzyme. The clotting of shed blood is regarded as an adaptation of nutritive processes in defence against hæmorrhage.

A controversy has long existed on the capacity of blood plasma to participate in immune reactions. Several investigations have been directed to ascertaining if the albumin-globulin complex of serum, called " complement " (which contributes to immune reactions) exists in normal plasma. Metchnikoff (1895) believed that this material is present in leucocytes, but not in plasma. Gengou (1901) suggested that the leucocytes are damaged by blood clotting and then yield complement. Gurd (1912) maintained that an antecedent body (" complementogen ") exists in plasma and is converted into active material after the shedding of blood. Addis (1912) observed in bird's plasma, just before clotting occurred, the kind of hæmolysis which requires the presence of complement. He concluded that plasma contains complement. But in these experiments the time interval between the occurrence of hæmolysis and coagulation was so short that proof was not furnished of the presence of complement in unaltered plasma. The complement might have been produced by the changes in plasma which

precede coagulation. The pure blood of the domestic fowl when centrifuged in a paraffined tube under oil at 3°–4° C. provides, however, a plasma free from cells and their *débris* which remains completely fluid at 38° C. for several hours. This plasma rapidly gives the reactions which are generally accepted as indicating the presence of complement, of agglutinin and of natural hæmolysin. The plasma of the bird possesses all the qualities necessary for the occurrence of immune phenomena, and it seems probable that mammalian plasmas are similarly endowed (Pickering and Taylor, 1927).

In the chapters devoted to abnormal changes in circulating blood much evidence is cited which suggests that reactions in plasma control the response of the body to certain toxic substances, determine the behaviour of blood platelets, alter the condition of the capillaries, and possibly produce autolysis in the liver. It seems evident that many of the functions of plasma are so constantly exercised that their existence is disclosed only when the routine of life is deranged, either by experimental interference or by morbid processes.

The Hereditary Transmission of certain Conditions of Blood Plasma

The hereditary character of the capacity of iso-agglutination in human bloods and the transmission of this quality in accordance with Mendel's Law is now established (von Dungern and Hirschfeld, 1910; Learmonth, 1920) and is illustrated by family blood groups. The observations just cited show that pure plasma possesses the power of agglutinating heterologous blood corpuscles. Reviewed together, these facts suggest that plasma possesses hereditary properties. Other facts pointing in this direction will be cited in the discussion of the condition of the blood of abnormal persons. In hæmophilia it appears that the hereditary quality is an increased stability of plasma complexes.

CHAPTER III

Fibrinogen

Definition and occurrence—Origin—Method of separation from body fluids—Purification *in vitro*—Behaviour *in vivo*.

FIBRINOGEN exhibits the general characteristics of globulin, with one important difference. It is clotted by thrombin and is thus distinguished from all other fractions of plasma protein. From these salient features, fibrinogen may be provisionally defined as globulin that is coagulable by thrombin. It will, however, be seen later that this definition may apply only to the fibrinogen of body fluids and not to fibrinogen divorced from calcium and sodium ions. The intravascular coagulants of tissue extracts were named "tissue fibrinogens" by Wooldridge (1886–1893), and this nomenclature has been adopted by Mills and his collaborators (1919–1926). These substances are protein-phospholipin complexes and thus differ chemically from fibrinogen. They are not clotted by thrombin. Moreover, the clots formed by their action probably have not the same chemical composition as fibrin. This nomenclature is, therefore, inappropriate.

Sufficient fibrinogen for the complete coagulation of the blood can be obtained from the post-embryonic blood plasmas of normal vertebrates and from almost all abnormal bloods, including that of hæmophilics (Wöhlisch, 1923). In certain rare cases of hæmorrhagic disorder fibrinogen may be absent, or almost absent, from blood plasma. In such cases excessive bleeding occurs from wounds and may be mistaken for that found in hæmophilia (Morawitz, 1923). Fibrinogen may be locally destroyed by the action of either streptococci or staphylococci and the blood so rendered incoagulable.

Fibrinogen is obtainable from lymph, chyle, pericardial fluid, and from many, but not all, pathological transudates. It is said to be present in bone marrow (Müller, 1905), and in the secretion of the vesiculæ seminales of the guinea-pig (Landois, 1888).

The Origin of Fibrinogen

Many facts point to the formation of fibrinogen in the liver. Blood clotting is suppressed after hepatic necrosis (Lidbeck, 1845; Ansiaux and Corin, 1894), and this is accompanied by a marked decrease of fibrinogen (Jacoby, 1900; Schultz and collaborators, 1925). A loss of fibrinogen occurs in cirrhosis of the liver, after the hepatic lesions of chloroform poisoning (Whipple, 1913), in acute atrophy of the liver (Foster, 1924) and during extensive tubercular destruction of that organ (McLester and collaborators, 1924). Exhaustive experiments on the variations in the amount of fibrin obtainable from blood after changes in diet, transfusion, hæmorrhage, plasma depletion, cell injury, intoxication, inflammation, liver injury and an Eck's fistula also point to the same conclusion (Foster and Whipple, 1921, 2). The conclusiveness of such evidence is questioned by Mathews (1926), on the grounds that other tissues that produce fibrinogen may also be injured. The views of clinicians are, however, confirmed by those of physiologists. An almost complete loss of fibrinogen occurs after the occlusion of the hepatic blood vessels with paraffin wax and after the extirpation of the liver (Doyen and collaborators, 1905; Nolf, 1905, 1927).

Less is known of the alleged subsidiary sources of fibrinogen. From its presence in bone marrow and from its increase in the plasma during leucocytosis, Müller (1905) claims that the bone marrow is a producer and suggests accessory production in the spleen. The presence of a substance in an organ does not afford proof of its origin in that organ. Two facts oppose these suggestions, the absence of any decrease of fibrinogen in aplastic anæmia (Whipple, 1914) and after splenectomy. The influence of the pancreas on the liberation of fibrinogen (or of its mother substance) from the liver seems, however, probable from the observations

of Wohlgemuth (1917) and of Hiruma (1923). There is also some evidence that a small amount of fibrinogen is stored in the intestines and can pass again into the circulation (Foster and Whipple, 1921, 2, 4).

There remains the possibility of the formation of fibrinogen in the blood itself or by the vascular endothelium. Several speculative suggestions have been made. Of these only two demand notice. Mathews (1900) suggests that fibrinogen is secreted by leucocytes: Herzfeld and Klinger (1920) maintain that it is formed in the plasma from raw material provided by the disintegration of both leucocytes and blood platelets. The former suggestion is improbable, since, firstly, an increase in the number of leucocytes is not associated with any increment of fibrinogen (McLester and collaborators, 1925) and, secondly, an increased amount of fibrin is obtainable from blood when the leucocytes are diseased (Pfeiffer, 1897). The latter suggestion gains some support from the increase of fibrinogen during the lysis of leucocytes, but lacks proof.

There are, however, several indications that the products of the lysis of erythrocytes participate in the formation of fibrinogen. In immunised horses, Reymann (1924) finds a very close connection between the decrease in the number of red corpuscles and the increase of the fibrinogen-globulin complex of plasma. He also finds that substances which destroy erythrocytes, such as staphylolysin and pyrodin, cause an increase of fibrinogen, followed by an increment of globulin. According to this observer, the protein of erythrocytes is converted into that of plasma; but it is equally probable that the products of hæmolysis merely stimulate the production of fibrinogen in other parts of the body and are not actually transformed into fibrinogen. The statement of Levi-Crailsheim (1923) is more definite, but, unfortunately, lacks confirmation. He avers that the remnant of protein attached to bilirubin is converted by the liver into fibrinogen and passes from thence into the circulation. This remnant of protein is said to be a converted form of globin and thus to be derived from hæmoglobin. Other inferences point in a similar direction. From a comparison

of the precipitability and antigenic properties of the fibrinogens of animals of different species, Davide (1925) also concludes that the raw material of fibrinogen exists in erythrocytes, and that species-variations in the properties of fibrinogen are due to different degrees of differentiation from the protein of stromata. The original memoir should be consulted ; it provides forcible evidence in support of the views of its author. It has, however, been shown that there is no conclusive evidence of the transformation of one fraction of protein into another.

Methods of Separation from Body Fluids

Two methods are in use in the preparation of fibrinogen, namely, precipitation and re-solution by neutral salts (Hammarsten, 1879, 1914 ; Reye, 1898), and precipitation by slight acidification after dilution with water (Schmidt, 1879 ; Mellanby, 1909, 1). The latter procedure has been adversely criticised, owing to the fibrinogen so obtained being associated with other globulins (Hammarsten and Hedin, 1914). Both methods yield, however, a complex of globulins and not a single substance. The method of Hammarsten may be described as follows :—

Three volumes of blood are shed directly into one volume of a saturated solution of $MgSO_4$, the mixture being constantly stirred. The erythrocytes and leucocytes are removed, either by filtration or by centrifuging. The salted plasma so obtained is precipitated by the addition of an equal volume of a saturated solution of NaCl. The precipitate is rapidly pressed between filter papers, is re-dissolved in an 8 per cent. solution of NaCl, and is again precipitated by an equal volume of a saturated solution of that salt. Repetition of this process, at least three times, is necessary when a product approximating to purity is required. The fibrinogen, if finely divided, dissolves in water, owing to a small amount of NaCl entangled in the precipitate. Further purification is effected by precipitation with twice its volume of saturated NaF, re-dissolving in a 0·5 per cent. solution of NH_4OH and neutralising. To the final product, a 1 per cent. solution of NaCl is added to facilitate solution (Hammarsten and Hedin, 1914).

It frequently happens that the second and third precipitates will only partially dissolve. A solution can be obtained, how-

ever, by adding a few drops of a 0·5 per cent. solution of sodium bicarbonate. The final precipitation with NaF should be omitted, since part of the protein remains insoluble. Purification by adsorbents should be substituted.

Two improvements in Hammarsten's technique should be generally adopted. Blood should be shed through a paraffined cannula, and subsequent operations should be conducted at 3°–4° C. The former procedure prevents contamination with tissue juices, the latter partly inhibits changes in the plasma.

A soluble oxalate or citrate may be used in place of $MgSO_4$, only sufficient for the prevention of clotting being commonly used: viz., slightly over 0·1 per cent. of sodium oxalate, or 0·25 per cent. of sodium citrate. Such solutions are hypotonic to blood and are said to alter the plasma. Isotonic solutions, therefore, are recommended; namely, either a 1·55 per cent. solution of sodium oxalate, or a 3·5 per cent. solution of trisodium citrate (Leendertz and Gromelski, 1922). Using tests based on the antigenic properties of fibrinogen, Davide (1925) could not, however, detect any difference in the behaviour of fibrinogen obtained when isotonic and hypotonic sodium oxalate were used respectively. Sodium citrate is preferable to sodium oxalate, since the latter salt causes some precipitation of the plasma (Leendertz and Gromelski, 1922). Experiments on the anticoagulant action of these salts *in vivo* also indicate that in some animals, particularly in the cat, there is less disturbance of the plasma by citration than by oxalation (Pickering and Hewitt, 1925).

The use of $(NH_4)_2SO_4$ in the preparation of fibrinogen was first described by Reye (1898), and was popularised by McLean (1920).

To an oxalated or citrated plasma is added one-fourth of its volume of saturated $(NH_4)_2SO_4$. The precipitated protein is removed by centrifuging, and is thrice washed by layering over with a one-fifth saturated solution of $(NH_4)_2SO_4$. It is then dissolved in a 2 per cent. solution of NaCl equal in volume to that of the original plasma. Partial purification is effected by filtration, followed by the repetition of the precipitation and washing described above. The precipitate is finally dissolved in a 1 per cent. solution of NaCl, and when filtered is ready for use (McLean, 1920). This process is useful for demonstrations,

since the precipitates are readily soluble. In research its value is dubious, as a series of solutions exhibiting constant qualities cannot be obtained.

The baneful influence of high concentrations of salts on protein was rightly emphasised by Mellanby (1909, 1), who utilised the discovery of Delezenne (1897) that the blood plasma of birds remains fluid at room temperatures without the addition of anti-coagulants. The fraction of protein containing fibrinogen was precipitated by the modification of Schmidt's technique described below.

The plasma of a domestic fowl is diluted with 15–20 volumes of distilled water, and the fraction of protein including fibrinogen is precipitated by adding a few drops of a 0·1 per cent. solution of acetic acid. The precipitate is ground up in distilled water and diluted with water so that the suspension is equal in volume to that of the original plasma (Mellanby, 1909, 1). Material free from the products of the artificial lysis of the formed elements of the blood (including thrombocytes) can readily be obtained if the blood is shed through a paraffined cannula, and is centrifuged under oil in a paraffined tube at the temperature of an ice-chest (Pickering and Reeves, 1925).

This process does not give satisfactory results when applied to the blood of cats or rabbits. Although unclotted plasma can be obtained and manipulated at the temperature of an ice-chest, the precipitate formed by acidification is only partly soluble in a 1 per cent. solution of NaCl, even after the addition of an appropriate amount of alkali. The "denaturation" of protein is thus greater than in salting-out. An almost completely soluble product can be obtained, however, when the cooled plasma of the horse is used. Dale and Walpole (1916) tested the value of the preparation of fibrinogen by the acidification of the oxalated plasma of the rabbit. A considerable amount of "denaturated" protein was found.

The Purification of Fibrinogen

Several attempts have been made to purify fibrinogen by adsorption. Dale and Walpole (1916) exposed the oxalated plasma of the rabbit to a well-shaken suspension of $BaSO_4$. The mixture was thoroughly stirred and allowed to stand for several hours. The $BaSO_4$ was removed by centrifuging. Fibrinogen was obtained by using a slight modification of Hammarsten's method. It remained fluid indefinitely when

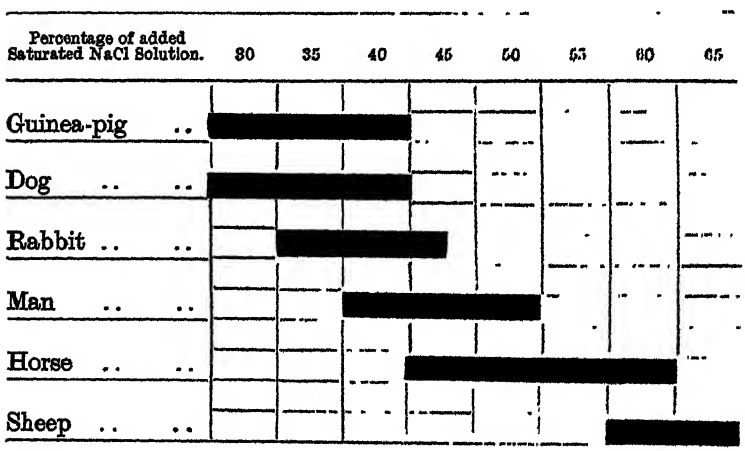
kept cold. A similar technique was used by Bordet (1919), the adsorbent being $Ca_3(PO_4)_2$, which had been precipitated from an acid solution by adding ammonia. After adsorption by this material, the fibrinogen remains fluid at room temperatures. Both these preparations clot on incubation at 38° C. if a few drops of a dilute solution of $CaCl_2$ are added (Sumner, 1922). Contamination with prothrombin is thus clearly indicated. A more adsorbent suspension of $Ca_3(PO_4)_2$ was obtained by Mills and Mathews (1924) by neutralising an acid solution of that salt with NaOH. By its use fibrinogen was almost divested of prothrombin. Greater success attended the work of Sumner (1922). Oxalated plasma was shaken with a 10 per cent. suspension of animal charcoal, and the fibrinogen was subsequently extracted by Hammarsten's method. It is said that the fibrinogen is completely freed from both prothrombin and the products of the disintegrations of blood platelets. Great difficulty was experienced in obtaining an adequate supply of charcoal that adsorbed the latter material. These experiments were repeated by the present writer. In four cases a purified fibrinogen was obtained that remained unclotted for a fortnight, after adding an appropriate amount of $CaCl_2$ and continuous incubation at 38° C. In ten other experiments partial clotting occurred, after incubating in sterile tubes for two or more days. The stable product coagulated on heating to 56°–60° C., leaving some uncoagulated globulin in solution. Like crude fibrinogen it yields, on cataphoresis, positively and negatively charged fractions. It is thus evident that the fibrinogen prepared by Sumner's method is a complex of globulins carrying different electrical charges. The extent of the alteration produced in this complex by adsorption is unknown. A considerable amount of material is lost, thus indicating partial disintegration.

The Properties of Fibrinogen *in vitro*

Prior to the work of Sumner, all investigations on the qualities of fibrinogen were made on a complex including fibrinogen, prothrombin and material derived from blood

platelets. Many of the properties of this complex have already been mentioned, but for the convenience of the reader are now recapitulated, additional information being added: (i.) It exhibits the general characteristics of globulin. (ii.) It is clotted by thrombin. (iii.) It is also clotted by incubating at 38° C. after the addition of a trace of $CaCl_2$. (iv.) It is coagulated on heating to 56° C., yielding fibrin and a soluble globulin, providing the concentration of salts and hydrions is the same as in plasma. (v.) It is the most readily precipitable fraction of plasma protein (see Table I., p. 6), and is thus the least dispersed of these fractions. (vi.) When precipitated by water it forms translucent masses, which are white, tough and elastic. (vii.) This precipitate dissolves in a 5–10 per cent. solution of NaCl. Out of this solution it coagulates at 52°–55° C. (Davide, 1925).

TABLE II.—*Showing graphically the Concentrations of Common Salt within which lie the Upper and Lower Limits of Precipitation of different Fibrinogens on Salting out from 20 per cent. plasma.*



The complex containing fibrinogen exhibits little or no specificity in reacting with thrombin. Likewise, the fibrino-

gens of animals of different species exhibit no appreciable differences in precipitability from undiluted plasmas. When 20 per cent. plasmas are used, however, the differences are striking, as is shown in Table II., abridged from Davide's memoir.

These differences may be interpreted as due to species-variations in the stability of plasma complexes, which, on dilution, lead to different degrees of dispersion of the proteins. Specificity is also well marked in the capacity of crude fibrinogens to produce antibodies (Gengou, 1902), and the degree of specific action corresponds closely with the variations in precipitability illustrated in Table II. (Davide, 1925).

The coagulability of fibrinogen, after its purification by Sumner's process, was exhaustively investigated by Kugelmass (1922, 1923) and was compared with that of oxalated plasma. In both cases the coagulant used was a thrombin generated by mixing crude serum with tissue and cell extract. The use of crude serum was unfortunate, since it contains very variable amounts of prothrombin (Mellanby, 1909, 1). It is also unfortunate that control experiments were not made with a purified thrombin. Despite the difficulty in appraising the precise value of this work, it is evident that the salient properties of fibrinogen divested of prothrombin are almost identical with those of fibrinogen bound to prothrombin. Except in the production of thrombin, fibrinogen thus appears to be the predominant partner in this union.

According to Kugelmass (1923), the optimum hydrion concentration for the clotting of purified fibrinogen is close to true neutrality. This condition also holds good in the clotting of mammalian blood (Stuber and Heim, 1916), and in the coagulation of oxalated mammalian plasmas by certain phosphatides (Zunz and La Barre, 1921). Kugelmass also states that the range of hydrion concentration within which clotting occurs in both purified fibrinogen and oxalated plasma lies between *p*H5 and *p*H8, whilst variations on either the acid or alkaline side of neutrality delay clotting. This corresponds with the statement that the alkalinity of blood plasma tends to maintenance of its fluidity (Mellanby, 1909, 1), and with the finding that the

coagulant action of thrombin on crude fibrinogen is delayed when the medium is slightly alkaline (Herzfeld and Klinger, 1915). It is significant, writes Kugelmass, that the limits of hydrion concentration within which the clotting of whole blood and of oxalated plasma occur, comprise on the one side the isoelectric points of serum globulin (4·5) and serum albumin (4·7), and on the other side that of fibrinogen (8), whilst the optimum for clotting corresponds with the isoelectric point of fibrin (7·2). From these data, he concludes that the conditions necessary for the clotting of mammalian blood are : (i.) the proteins of serum must be on the anion side of their isoelectric points, and thus behave as hydrophile emulsoids, and (ii.) fibrinogen must be on the cation side of its isoelectric point, that is to say, when the hydrion concentration is greater than 1×10^{-8} it behaves like a hydrophobe colloid.

From the same observations, Kugelmass (1923) suggests that the coagulation of fibrinogen is due to the adsorption of fibrinogen on particles of thrombin. A similar view was suggested by Howell (1916) and is advocated by Hekma (1916–1927). It is based on the changes apparent when the formation of fibrin is viewed under the ultra-microscope. Nevertheless, Howell (1925, 1) questions both the accuracy of the work of Kugelmass and the adequacy of his conclusions. He states that fibrinogen, when dissolved in a 1 per cent. solution of NaCl, has an isoelectric point between 4 and 5, and that thrombin in aqueous solution has a similar isoelectric point. It is patent that these observations are opposed to the conclusion that the reaction of fibrinogen and thrombin is always due to the electrical union of oppositely-charged particles. There is, however, no doubt that Howell's thrombin differs materially from that used by Kugelmass, the former preparation being free from phosphatides, the latter containing them. The burden of proof that phosphatide-free thrombin exists in shed blood rests, however, on Howell. The difficulty in reaching conclusions when the behaviour of purified material differs substantially from that not purified is illustrated by the observation of Funk (cited by Howell, 1925, 1) that albumin and globulin, when purified and dialysed, precipitate fibrinogen, for no such reaction occurs in whole blood. Sufficient has been said to show that dogmatic conclusions on the clotting of fibrinogen

are unwarranted. The rapid adsorption of thrombin to the masses of fibrin in shed blood points to physical forces being important participants in clotting, but chemical changes may also be involved. Indications of the chemical action of thrombin on fibrinogen will be discussed in Chapter VI.

According to De Waele (1926, 1927), the fibrinogen of blood is a fibrinogenate of calcium, or more probably of calcium and sodium, the proportion of these cations being a function of the hydrion concentration. Two minima of solubility are exhibited, one near *p*H4, the other near *p*H7. The removal of CO_2 from plasma produces a hydrion concentration which tends to insolubility, and this is manifested by the gel-formation said to be characteristic of calcium complexes. Fibrinogen itself, in contra-distinction to its salts, is isoelectric at *p*H6·9, and is incoagulable by heat. It forms compounds with the alkali metals and with various acids, these bodies exhibiting varying degrees of solubility and of coagulability. When freed from lipoids, coagulation no longer occurs in ordinary conditions, but coagulability is restored by the addition of cephalin, peptone, or gelatin. It is claimed that these bodies favour the entrance of calcium ions into the fibrinogen complex.

Electrodialysed fibrinogen, when dissolved in 0·8 per cent. NaCl with a trace of Na_2CO_3 (*p*H of the solution 6·9), preserves its fluidity and transparency after the addition of electrodialysed thrombin. The adding of a drop of a 1 per cent. solution of $CaCl_2$ to 1 c.c. of the mixture produces flocculation. Undialysed thrombin, which contains calcium, flocculates electrodialysed fibrinogen and coagulates oxalated plasma. It appears that salts of calcium are essential for the interaction of thrombin and fibrinogen (Rabinovitch, 1926). A similar conclusion has been reached by Loucks (1926).

The Behaviour of Fibrinogen in Body Fluids

One of the landmarks in the history of blood coagulation is the observation of Fredericq (1878) that the plasma in the excised jugular vein of the horse coagulates at the same temperature (56° C.) as the fibrinogen prepared by Hammarsten. From these facts arose the belief that the fibrinogen

of circulating blood is in substantially the same condition as that known in the laboratory. The protocols of Fredericq's experiments reveal, however, that the plasma in excised veins undergoes rapid change, since a small clot and some thrombin are almost invariably present. These observations I have confirmed. They show that this plasma differs radically from that of normal circulating blood, which does not contain either clots or thrombin. The coincidence of heat coagulation is thus robbed of the significance commonly assigned to it.

Several observations establish that the association of fibrinogen with body fluids modifies its stability. Hammarsten (1879) found that the fibrinogen of hydrocele fluid coagulates at 56° C. only if a trace of thrombin be present. Howell (1916) observed that the fibrinogens prepared by a single precipitation from the relatively stable plasmas of birds and terrapins are often not clotted by thrombin, and this statement applies to the complex containing fibrinogen which is precipitated by acidifying the cooled plasma of the horse. Lisbonne (1924) noted that certain transudates, containing fibrinogen, are resistant to coagulation by the thrombin generated by mixing serum and tissue extract.

Fibrinogen always coagulates *in vitro* after heating to 56°–60° C. for two or three minutes, providing the concentration of salts and hydrions is the same as in blood plasma. Nevertheless, this is not true *in vivo*. When the blood in the freshly excised and pulsating heart of the cat, with its cavities unexposed to air, is heated for fifteen to twenty minutes to 56°–60° C., the fibrinogen remains uncoagulated and can subsequently be separated from the plasma by salting out. When, however, intraventricular plasma is disturbed by citration and "re-calcification," or when partial clotting has occurred, fibrinogen is destroyed at these temperatures (Pickering and de Souza, 1923). It is thus evident that the fibrinogen of undisturbed plasma behaves differently from that of plasma which has suffered change. This conclusion was endorsed by Fiessly (1925, 2), who, by the repetition of an almost forgotten experiment, devised by Hayem (1888), not only demonstrated that the

resistance of fibrinogen to coagulants is greater *in vivo* than *in vitro*, but also showed that the complex containing fibrinogen is so labile that stasis in a vein alters its stability. The following is the protocol of this experiment :—

A portion of a jugular vein of a dog was separated from the general circulation by double ligature. Ten c.c. of fresh serum were then injected into the saphenous vein and ligatures were subsequently placed on the corresponding part of the other jugular vein. Ten minutes later the animal was killed. The blood was fluid in both the general circulation and in the portion of the vein which was isolated before the injection of the serum ; that in the segment of the vein isolated after the injection of the serum was clotted. It may here be noted that the addition of fresh serum to shed plasma hastens its coagulation and that the condition of immobility *in vitro* favours the continuance of fluidity.

It has been amply demonstrated that large amounts of dried and re-dissolved thrombin can be injected into the circulation without causing intravascular clotting, and that the addition of similar amounts of the same kind of thrombin to either plasma or fibrinogen *in vitro* causes rapid clotting (Wooldridge, 1885 ; Mellanby, 1909, 1 ; Mills and Mathews, 1924). A still current explanation of these facts is the supposition that *in vivo* the thrombin is neutralised by the secretion of an excess of antithrombin, produced either by the liver (Howell, 1914 ; Gasser, 1917), or by the vascular endothelium (Popielski, 1913). In this explanation the possibility is ignored that fibrinogen exists *in vivo* as part of a larger complex which resists the action of thrombin. Several observations oppose the former view, but are consistent with the latter. Large amounts of thrombin can be injected into the circulation of animals deprived of hepatic activity, without causing intravascular clotting (Pickering and Hewitt, 1922, 2). In the following experiments the fibrinogen of blood plasma remained fluid in contact with blood clots under conditions which preclude the secretion of antithrombin. They also show that disturbance of plasma complexes is a pre-requisite condition for the clotting of fibrinogen by thrombin.

The blood contained in the excised jugular vein of an ox was transferred by Lister's method (1880) into a glass tube. The tube was sealed. After the expiry of twenty-four hours the corpuscles had sedimented and the bulk of the plasma was fluid, except for a thin crust of a clot adherent to the tube. The fluid plasma clotted in 1 min. 40 secs. when poured on a glass plate. It contained active thrombin, since 0·2 c.c. of the plasma rapidly clotted 0·5 c.c. of fibrinogen purified by Sumner's method. A control experiment showed that this fibrinogen remained fluid after the addition of $CaCl_2$ and incubation for ten days at 38° C In another experiment plasma, similarly obtained, was transferred by a paraffined pipette to a paraffined vessel, evaporation being prevented by a film of liquid paraffin. This plasma remained fluid for an hour after stirring with a paraffined rod, but clotted in two minutes after stirring with a glass rod. When evaporation was permitted, clotting occurred in 15–20 minutes.

CHAPTER IV

The Inception of Blood Clotting

Occurrence—Nomenclature—The *rôle* of blood platelets—The physical changes that maugurate clotting—The material of blood plasma—The *débris* of blood platelets, cells and tissues—Synthetic coagulants—Inception of blood clottmg in disease.

During the latter half of the last century two discoveries completely altered the outlook on blood coagulation. The first revealed the presence of platelets in plasma and showed that their disintegration in shed blood is closely connected with the inception of clotting (Hayem, 1878; Bizzozero, 1882, 1883). The second established the importance of the *débris* of cells and tissues in the natural coagulation of blood in wounds and showed that their introduction into the blood-stream may cause intravascular clotting (Foà and Pellacani, 1880; Wooldridge, 1886–1893; Halliburton, 1886–1895).

It will be shown later that the coagulants of blood platelets contain phosphatides, and that those of tissue juices are composed of protein bound to phosphatides. Similar properties are, however, possessed by less complex bodies, such as the protein-like substances formed from amino-acids by artificial synthesis (Pickering, 1896–7). The intravenous injection of silica sols also produces intravascular clotting, but this is probably due to damage to the vascular endothelium and not direct action on the blood itself (Gye and Purdy, 1922).

Many years earlier De Blainville (1834) observed that the injection of brain-substance, or of emulsions of it, into the blood-stream, causes intravenous clotting. The hastening of the coagulation of shed blood by the action of damaged tissues has also long been known. But these facts were not used in the explanations of blood clotting which were current prior to the work of Wooldridge. Intravascular coagulants are also obtainable from erythrocytes (Wooldridge, 1893).

Substances that either incite or assist blood coagulation are obtainable from widely different sources and are said to be present in the disintegration products of all animal cells (Schmidt, 1892).

Similar bodies are present in some vegetable extracts, such as those of yeast and of certain fungi (Nausenbach, 1882 ; Grohmann, 1884). They are also formed by the growth of some micro-organisms (Loeb, 1903). In clotting by staphylococci, fibrinogen is first coagulated and then destroyed. This may occur without the production of thrombin, but in some cases the bacteria may act like thrombin, or by converting prothrombin into thrombin (Gratia, 1919, 1, 2, 1921). With staphylococcus aureus there is no formation of thrombin, but clotting is accompanied by an alteration in the hydrion concentration of the plasma (Cekada, 1926).

Other substances hasten clotting by augmenting the action of natural coagulants. Included in this group are certain proteoses, peptides and amino-acids, as well as " lecithin " and allied substances (Zunz and Györgi, 1920 ; Zunz and La Barre, 1921, 1, 2).

A hastening of the clotting of shed blood is also caused by chemically inert substances which are wetted by blood. The presence of such bodies in the blood-stream produces, however, variable results. Although sclerotic accretions in the vascular system are often associated with clots, they may be present without causing thrombosis, whilst small crystals equal in size to erythrocytes (Pickering and Hewitt, 1922), finely powdered quartz (Tait and Eldridge, 1926), and even strings (Barrett, 1924), can be introduced into the circulation without causing intravascular clotting.

The Nomenclatures in Use

Much confusion of ideas has arisen from the many terminologies in use in the literature of blood coagulation. Different names have been given to the same material, whilst different meanings have been assigned to the same term. Clarity has been sacrificed to the desire of individuals for a nomenclature consistent only with their own theories.

The *débris* of leucocytes and platelets was named " thrombokinase " by Morawitz (1903), as he believed its action in blood coagulation is similar to that of a kinase. The participant in coagulation present in tissue extract was called " cytozyme " by Fuld and Spiro (1904), thus implying that it is an enzyme. This term is used by the important

group of investigators who follow the teaching of Bordet, but "cytozyme" is defined as a substance present in the disintegration products of blood platelets, cells and tissues that unites with a derivative of plasma to form thrombin (Bordet and Delange, 1912). The description of cytozyme as an enzyme is also rejected by Howell (1911–1926), the term "thromboplastin" being substituted. Unfortunately, this term is commonly used by the makers of various proprietary hæmostatics and, for this reason, I reluctantly discard it.

The describing of the coagulants of cells and tissues as "tissue fibrinogen" has already been criticised. These substances have also been called "nucleo-albumins" (Halliburton, 1886; Pekelharing, 1892) and "tissue coagulins" (Loeb, 1903). In the earlier literature, the plasma constituent that produces thrombin in shed blood is named "prothrombin" (Schmidt, 1892; Pekelharing, 1892). The word "thrombogen," suggested by Morawitz (1903, 1, 2, 1904), is not exactly synonymous with this term. By the action of material derived from the formed elements of the blood, thrombogen is said to be converted into α and β prothrombin. The former product is stated to form thrombin in the presence of calcium ions, the latter to remain inactive in serum. It is now known that thrombin loses its activity in serum, and there is no need to distinguish two kinds of prothrombin. "Plasmozyme" is the name given by Fuld and Spiro (1904) to the plasma constituent, which is said to be converted by cytozyme and calcium ions into thrombin. The term "prothrombin" was re-introduced by Howell (1910–1926), but is defined as a component of plasma that can be converted into thrombin by calcium ions alone, without the intervention of any other agent. The word "serozyme" is used by Bordet and his collaborators (1912–1920) to designate the material in serum, which they believe unites with cytozyme to form thrombin. An inactive form of serozyme is said to exist in plasma and is called "prosserozyme." A different nomenclature is used by Nolf (1908–1927). The names "thrombozyme," "thrombogen," and "fibrinogen" connote fractions of plasma protein which are said to combine and so form clots.

The term "thrombocyte," introduced by Dekbuyzen (1901) to describe the spindle-shaped cells found in the blood of amphibians, is now frequently used for all formed elements of the blood which are smaller than erythrocytes. It includes the blood platelets of mammals and the small nucleated corpuscles of birds and lower vertebrates.

The rôle of Blood Platelets (Thrombocytes) in the Inception of Clotting

Two opinions are current as to the mode of action of platelets in blood coagulation: (1) That their lysis liberates a substance essential for the formation of thrombin (Morawitz, 1904; Fuld and Spiro, 1904; Bordet, 1920; Stuber and Sano, 1923). (2) That blood coagulation is caused primarily by the breaking down of platelets and secondarily by a reaction in which the disintegration products of platelets participate (Cramer and Pringle, 1913). All these investigators agree, however, that the lysis of platelets is an essential occurrence in the spontaneous clotting of blood. The most cogent evidence supporting this conclusion is provided by the experiments of Cramer and Pringle (1913). They showed that the oxalated plasma of mammals when deprived of platelets by passage through a Berkefeld filter does not usually clot on recalcification at room temperature, but coagulates readily on the subsequent addition of platelets. It has also been demonstrated that frog's blood does not clot spontaneously when its corpuscles have been removed by filtration through vegetable charcoal, and a similar loss of coagulability occurs when frog's blood is almost deplateletised by the intravascular injection of relatively large amounts of finely divided particles of Indian ink (Tait and collaborators, 1926, 1, 2). These results seem conclusive to Tait and Green (1926). They suggest that platelets contain all the material necessary for the production of thrombin and that the word "prothrombin" might well be replaced by the phrase "unruptured thrombocytes."

It is unfortunate that these experiments are indecisive. Passage through a Berkefeld filter exposes the plasma to adsorbent surfaces and the intravascular injection of finely

divided particles also subjects the plasma to adsorption. Oxalated plasma, when exposed to adsorbents, loses sufficient of its prothrombin to remain fluid indefinitely at room temperatures after recalcification (Bordet and collaborators, 1912–1920). Besides this loss, partial disintegration of protein molecules probably occurs, since Zunz and La Barre (1921) found that the addition of certain amino-acids or peptides to plasma, after passage through a Berkefeld filter, causes clotting. Finely divided particles of Indian ink are excellent adsorbents. Vegetable charcoals are even more disruptive, since they also remove much of the fibrinogen from plasma (Sumner, 1922). Moreover, oxalated plasmas which have been deplateletised by filtration at different speeds, through animal charcoal, may remain fluid indefinitely at room temperatures after recalcification, but clot on incubation at 38° C. at different speeds—the time taken varying from an hour to four days. Exactly parallel phenomena occur when the fibrinogen-prothrombin complex is exposed to adsorbents, the time of clotting at 38° C. depending on the amount of prothrombin removed. It is thus probable that in the removal of platelets from blood, by the methods just described, a sufficient amount of prothrombin is abstracted from the plasma to prevent spontaneous clotting at room temperatures.

Furthermore, Mills (1927) states that when platelets are obtained free from contamination by tissue juices and by the *débris* of other blood corpuscles they do not clot purified fibrinogen, even after substances which convert prothrombin into thrombin have been added. He concludes that platelets do not contain either prothrombin or thrombin but, like tissue juices, they contain material which reacts with prothrombin.

There is a considerable amount of evidence that in certain conditions of both mammalian and bird's blood the thrombocytes are of less importance than other participants in clotting. The oxalated plasma of mammals killed at the height of digestion clots on recalcification almost as readily after the removal of blood platelets by passage through a Berkefeld filter as when unfiltered (Cramer and Pringle,

1913). This is also true of plasmas deprived of platelets by filtration through animal charcoal. It is thus evident that the products of digestion may take the place of the disintegration products of platelets in the inauguration of clotting. That these coagulants are either amino-acids or peptides seems probable from the already mentioned observation that such substances clot deplateletised plasma. An important subsidiary mechanism for the arrest of hæmorrhage exists in animals that do not commonly fast.

Other evidence pointing in the same direction is summarised below :—

It is stated that citrated rabbit's plasma slowly clots on recalcification after all its platelets have been removed (Roskam, 1926). (1) The intravascular injection of various substances, such as gelatin, "peptone" and neutralised thymus nucleic acid, may cause a temporary disappearance of platelets from the bloodstream, without markedly decreasing the coagulability of the blood (Achard and Aynaud, 1908, 1, 3, Sacerdotti, 1908, Roskam, 1922–1923; Pickering and Hewitt, 1924). The platelets are not destroyed, but are removed from circulation by aggregation in the capillaries (Roskam, 1922–1923). (2) When blood clots in a moist chamber on a vaselined surface, coagulation commences independently of the platelets (Achard and Aynaud, 1908, 2). (3) A like occurrence may sometimes be seen when blood, after the addition of nicotine, is viewed under the ultramicroscope. The needles of fibrin formed either may commence from agglutinated platelets, or may appear diffused in the plasma without the agglutination of platelets (Mangold and Kitamura, 1924). (4) There is no constant relationship between substances that inhibit the lysis of platelets and those which delay the inception of blood clotting (Pickering, 1925). (5) The blood plasma of the domestic fowl, when divested of all formed elements prior to their lysis, clots after severe shaking, and after incubation in a sterile tube for ten hours or longer. The addition of a few drops either of thrombocyte-extract, or of tissue extract produces clotting in a few minutes (Pickering and Reeves, 1925, 1, 2). All the materials necessary for clotting are thus present in the plasma of the bird, but the intervention of products derived from either cells or tissues is essential for a speed of coagulation sufficient for the control of hæmorrhage.

The evidence provided by pathologists is not so varied, but it is also suggestive. In purpura hæmorrhagica the number of platelets in blood exhibits no relationship to its coagulability (Pratt, 1906). A fall from 300,000–400,000 per c.mm. to less

than 10,000 per c.mm. may occur without any change in the speed of clotting (Roskam, 1922–1923), whilst cases of pernicious anæmia have been recorded in which there is said to be a complete absence of platelets in blood that clots rapidly when shed (Ehrlich, 1924).

In hæmophilic blood the platelets are more stable than in normal blood (Howell, 1926, 2), but such blood may remain fluid at room temperatures after the complete lysis of platelets by the addition of saponin.

Despite these facts, platelets are usually an important factor in clotting. Their partial removal from oxalated plasma delays clotting after recalcification (Mosen, 1893), and the subsequent addition of disintegrating platelets, or extracts of them, to such plasma hastens its coagulation (Bordet and Delange, 1912). Similar phenomena occur when cat's plasma, obtained by centrifuging cat's blood in a paraffined tube at 3°–4° C., is substituted for oxalated plasma.

The fluidity of pure bird's blood *in vitro* is largely due to the stability of its thrombocytes at room temperature. At 30°–40° C. the thrombocytes disintegrate on surfaces wetted by blood, and release a coagulant possessing the properties of cytozyme. The thrombocytes of the bird are, therefore, important participants in the arrest of internal hæmorrhages and of bleeding on hot skin. The juices of the damaged tissues of birds (domestic fowl and duck) contain an active coagulant and also rapidly disintegrate thrombocytes, even at room temperatures.

The Physical Changes that Inaugurate Clotting

In mammalian blood the lysis of platelets is not the primary cause of spontaneous clotting. Prior change in the plasma is an essential occurrence. Several facts support these statements.

White thrombi composed of disintegrated platelets can form naturally in blood vessels without causing clotting in the adjacent blood. (The blood retains its capacity to clot when shed.) Similar localised thrombi have been produced experimentally in the right ventricle and pulmonary artery by the intravascular injection of tissue extracts (Gutmann, 1914; Burke and Tait, 1926). The intravenous injection of saponin into anæsthetised cats completely disintegrates the platelets without causing intravascular clotting. Similarly, if saponin is mixed with blood

shed under oil on to paraffin, the platelets are destroyed, but the blood remains fluid at room temperatures for an hour or longer. Stirring with an oiled rod does not provoke clotting, but stirring with a glass rod causes rapid coagulation.

The observations just described show that physical changes in the plasma are a necessary prelude for the interaction of blood plasma with the *débris* of platelets. In like manner, physical rather than chemical action is the cause of the lysis of thrombocytes on surfaces that are wetted by blood, since their disintegration is more rapid on chemically cleaned glass than on glass not so clean (Tait and Green, 1926). Each of the initial steps in the clotting of blood is, therefore, a physical process.

The physical aspects of blood coagulation have attracted the attention of several writers. Bordet and his collaborators (1912–1920) state that the first change in blood shed upon a surface which it wets is due to contact-action converting an inactive constituent of the plasma (prosero-zyme) into a chemically active substance (serozyme). The only explanation given of the physical forces involved is that adsorption by the wetted surface concentrates and renders active the inactive material (Bordet, 1919). Nolf (1908–1922) distinguished "thromboplastic substances," which act as physical disturbants of the plasma, from plasma constituents that unite in the formation of clots. In the former category are included tissue juices and bodies wetted by blood. From observations on the influence of changes in the pressure of mixtures of O_2 and CO_2 on the speed of the clotting of blood shed on paraffined surfaces, Chio (1917) infers that the tension of CO_2 in plasma affects the equilibrium of the calcium salts and so regulates fluidity and clotting. The recent work of Baumberger (1926) lends some support to this view. But shed blood slowly clots on water-wettable surfaces when the gaseous tensions of the plasma are preserved unchanged. Furthermore, blood circulating through a collodion tube connecting an artery and a vein forms thrombi composed of platelets, and subsequently clots (Shionoya and collaborators, 1927). According to Herzfeld and Klinger (1917–1920), the chief factor in

blood clotting is an alteration of the dispersion of plasma protein, whilst Hekma (1916–1924) attributes fibrin formation to a change from a state of emulsion to one of true suspension followed by the aggregation of particles. Bloch (1920) affirms that contact of the blood with air, dust and so-called thromboplastic material converts "inactive calcium" into a precipitant of plasma colloids, but experimental evidence in support of this suggestion appears to be wanting.

Several writers have noted the similarities of the phenomena of blood clotting to those of precipitation. Howell (1916) showed that in the action of thrombin on fibrinogen the formation of a precipitate or of a gel depends on the electrical condition of the fibrinogen. Zunz (1922) observed that the addition of cytozyme to fibrinogen causes flocculation, whilst Reeves and I found that the production of a slight precipitate in pure bird's plasma often induces gel-formation. Bearing these facts in mind, the electrical changes produced by the contact of blood with a surface which it wets seem sufficient for the inauguration of clotting. A wetted surface becomes electronegative, and the presence of a negative charge implies the existence of an equal positive charge in the liquid in contact with it, as the other component of the Helmholtz double layer. Some of the colloidal complexes of plasma are electronegative, since the clotting of circulating blood in an electric field takes place at the pole which accumulates negative charges (Lusk, 1912), whilst serum is electrically inactive (Hardy, 1903; Robertson, 1918). A change in electrical conditions is thus a feature of normal clotting. Ions of the opposite sign to that of the substance precipitated act as precipitating agents (Loeb, 1921). The electrical conditions requisite for precipitation thus exist when blood is in contact with a surface which it wets and clotting results. Like conditions are present in blood in contact with wounds, whilst the juices of damaged tissues disturb the plasma by their hydrophile qualities and by alterations in the distribution of electrolytes (Pickering and Hewitt, 1921). A similar explanation seems applicable to the lysis of platelets on surfaces wetted by blood.

contains all that is necessary for its clotting. By cooling the oxalated plasma of a horse to 0° C. for two days, Nolf (1921, 1, 1922) obtained a protein precipitate which is insoluble at 0° C., but soluble at 37° C. (This is the material called "thrombozyme"). It reacts, says Nolf, with the prothrombin of mammals, but not with that of birds or fishes, and thus differs from the cytozyme of blood platelets, which does not exhibit specificity in reacting with thrombin. It is insoluble in alcohol and again differs from cytozyme. It was not apparently tested for phosphorus. The pure plasma of the domestic fowl (deprived of all formed elements prior to their lysis) also yields, when similarly treated, a precipitate possessing the solubilities of thrombozyme. This body gives the xanthoproteic, biuret and tryptophane reactions and contains phosphorus. When added to the plasma from which it has been precipitated, it slowly causes clotting. It is not an enzyme, since its activity is retained after heating to 100°–120° C. All that is necessary for clotting therefore exists in bird's plasma (Pickering and Reeves, 1925, 2).

The following experiment shows that thrombin is obtainable from the deplateletised plasma of the cat :—

Blood is shed from an anæsthetised fasting cat through a paraffined cannula into isotonic sodium oxalate. It is centrifuged in an ice-chest, twice filtered through animal charcoal, and the plasma so obtained is recalcified. (This plasma does not clot spontaneously at room or body temperature and does not contain blood platelets.) It is shaken with an equal volume of chloroform for an hour in a sealed tube. The chloroform extract, on evaporation to dryness, yields material containing protein which gives the distinctive reactions of thrombin ; that is to say, it clots both oxalated plasma and fibrinogen purified by Sumner's method.

Material capable of generating thrombin, therefore, exists in deplateletised plasma, but it is so firmly bound to other plasma complexes that it remains inactive until released by some extraneous agency. Among natural bodies, crude cephalin is active in this respect, for it has been shown by Mills and Mathews (1924) that plasma which has almost

been deprived of coagulants by adsorption clots slowly (in sixteen hours) at room temperatures after the addition of " cephalin." At body temperature this releasing action is rather more active, since plasma which has been rendered almost incoagulable at room temperatures by adsorption with animal charcoal clots in one to seven hours after incubation with cephalin. Most tissue juices contain crude cephalin. Their coagulant action may be due partly to the cephalin releasing the natural coagulants of the plasma from bondage. A more important reaction is the complete release of prothrombin when fibrinogen is clotted. Thus, in normal blood coagulation the stable reserve of prothrombin becomes rapidly available for a further production of thrombin.

The origin of the phosphorus-containing material of blood plasma is unknown. Possibly it is derived from the natural lysis of the formed elements of the blood. The speculations on the origin of prothrombin do not appear to be sufficiently conclusive to necessitate detailed discussion. Workers in this, domain should, however, consult the papers of Nolf (1906, 1921, 2), Bayne-Jones (1912), Bordet and Delange (1912), Drinker and Drinker (1916), Hurwitz and Drinker (1918), Tait and Green (1926), and Mills (1927).

The débris of Blood Platelets, Cells and Tissues

Disintegrating platelets are usually obtained by centrifuging oxalated plasma. The uppermost layer of corpuscles contains the platelets. When transferred to distilled water or isotonic saline they completely disintegrate. The particles should be thrice washed in isotonic saline, since oxalates slightly inhibit the onset of blood clotting (Stuber and Sano, 1923). The centrifuging of blood, shed through a paraffined cannula, in a paraffined tube at 3°–4° C., gives a better preparation of platelets.

The *débris* of platelets is soluble in alcohol, toluol and benzene, but is almost insoluble in acetone. Since these solubilities are exhibited by the lecithins, Bordet and Delange (1913) suggest that the cytozyme of platelets is allied to such bodies. A similar conclusion was reached by Hirschfeld and Klinger (1913), but is disputed by Howell (1912,

and thirteen phosphatide molecules. Partial disintegration by fat solvents leaves each fraction with only a small part of its original activity; complete disintegration yields 41 per cent. of cephalin and an anti-coagulant globulin. Most of the cephalin is said to be bound firmly to the protein molecule, and whilst in this condition to have no power of participating in the formation of thrombin. A small amount of cephalin is described as "free," and is believed to combine with prothrombin to form thrombin. The chemical constitution assigned to lung extract should be regarded as provisional, for, as Howell (1925, 1) points out, it is based on analyses of imperfectly purified material. It seems established, however, that in tissue extracts protein is bound to phosphatide material, and that the united material is more effective in promoting clotting than is phosphatide alone.

Several facts discredit the suggestion that the active material of blood platelets, cells and tissues is an enzyme. The *débris* of platelets can be heated to 100° C. without losing its activity, whilst the cytozyme in serum resists even higher temperatures (Sunzeri, 1923). Moreover, the cytozyme of platelets and of cells is apparently consumed in the production of thrombin (Bordet and Delange, 1912). Some indication of the nature of this reaction is afforded by the observation of Bordet (1919, 1920) that cytozyme unites in varying proportions with prothrombin, in a manner similar to union of toxins and anti-toxins.

An explanation is required, however, of the observation of Mellanby (1917) that in the production of thrombin from the fibrinogen-prothrombin complex the reaction is small at first, but proceeds with a constantly increasing velocity, in a similar manner to enzyme action. Mills (1926, 1) states that for every molecule of fibrinogen removed from plasma as fibrin, there are about eight molecules of prothrombin and cephalin liberated to form thrombin. It is this ratio of prothrombin to fibrinogen that is said to determine the cumulative character of the clotting process and simulate enzyme action. Although the precise proportion of prothrombin and fibrinogen molecules (or aggregates) may not be in the ratio suggested, there seems no doubt that the clotting of fibrinogen releases prothrombin from plasma, and so progressively increases the production of thrombin.

Another explanation of the behaviour of cytozyme in clotting is advocated by Howell (1911–1926). He supposes that the cephalin in this material in some way neutralises certain inhibitory substances, which he believes are available in blood and preserve its fluidity. He identifies the inhibitory bodies with heparin and antithrombin. It is difficult to accept this conclusion. Heparinised blood is much more stable than normal blood; it remains fluid at 40° C., and is not clotted by shaking with powdered glass. Moreover, it is stated that some antithrombins are not neutralised by cytozyme (Dale and Walpole, 1916; Gratia, 1921, 2). A recent attempt has been made, however, to harmonise the work of Howell with that of Bordet. Cytozyme, says Mills (1926, 2) reacts with prothrombin and forms thrombin, but also neutralises the antithrombin. He states, however, that the latter reaction occurs in serum, but not in unclotted blood. If this be true, the neutralisation of antithrombin has no relationship with the preservation of the fluidity of circulating blood.

The stability of bird's plasma *in vitro* has been explained as due to the presence of a large amount of antithrombin (Mellanby, 1909, 1) This plasma is clotted by chemically inert powders (Howell, 1925), and by shaking (Pickering and Reeves, 1925, 2). Mammalian plasmas (of the cat and rabbit) which have been stabilised by the addition of a minimal amount of Howell's antithrombin, do not clot when similarly treated. These facts seem irreconcilable with the explanation just cited. They are, however, intelligible by assuming that the colloids of bird's plasma are more firmly bound together than those of mammalian plasma, but can be dissociated by physical forces.

It is possible, however, partly to reconcile the conclusions of Howell with my own. In both outlooks, prothrombin and calcium ions are regarded as essential participants in the spontaneous clotting of blood, and the inactivity of prothrombin *in vivo* is explained by its association with stabilising material. Both writers believe that cytozyme modifies the condition of the stabilising material and so permits the formation of thrombin from prothrombin. But here our opinions diverge. It seems to me that cytozyme disturbs

produced a coagulant which is less active than the original extract. Furthermore, Vines (1921, 2) noted that the coagulant action of commercial lecithin (which contains cephalin) is augmented by the addition of tissue extract. This is ascribed to the added protein keeping the lipoid in a state of fine suspension, and not to a chemical reaction.

The Inception of Blood Clotting in Disease

The pre-clot changes in blood plasma during disease have almost escaped attention and should provide a fruitful field of research. Attention will be directed to the occurrence of such changes in anaphylactic and anaphylactoid phenomena and in intoxication by arsenobenzols. They are probably an essential feature in those allergic reactions which involve alterations in the stability of the blood. Variations in the condition of the participants in the inception of clotting seem also apparent in malaria from the occurrence of reactions similar to anaphylactoid phenomena.

The pre-clot changes in blood are inhibited in hæmophilia and similar changes probably take place in other hæmorrhagic disorders which exhibit delayed blood clotting. In normal blood the pre-clot changes are reversible (Pickering and Hewitt, 1921; Kugelmass, 1923), but it is not known if a like reversibility is a characteristic of morbid pre-clot changes. A decision on this question might prove of importance in therapy.

Observations on the speed of change of prothrombin into thrombin have been used in the investigation of hæmophilia and in differentiating some of the more obscure forms of purpura (Giffin, 1928). This method and determinations of the stability of the fibrinogen-prothrombin complex by its resistance to adsorption, should be employed in investigating the blood in the disorders named above as well as in fevers, and might provide a means of defining conditions which are still obscure.

CHAPTER V

The Stabilisation of Blood Plasma

The importance of the stabilisation of prothrombin—Antithrombokinase and antiprothrombin (heparin)—The conclusions of Bordet—Stabilisation by protective colloids—The hypothesis of Mills.

In a recent discussion of physiological equilibrium, Cannon (1926) suggests that the stability of body fluids *in vivo* is preserved by more complicated processes than those involved in simple physico-chemical reactions. This special form of equilibrium is designated "homeostasis," and it is suggested that any tendency to change is met by an increased effectiveness in the factor or factors which lessen change. "This postulate," says Cannon, "resembles the principle of Le Chatelier (1888), but differs in being conditional; in biology the organism may become not more but less resistant to a disturbing agent—*e.g.*, anaphylaxis." Other writers have recognised the possible application of Le Chatelier's theorem in the explanation of the resistance of blood plasma change (Pickering and Hewitt, 1922, 2), but a less generalised concept appears sufficient for the explanation of the stability of normal circulating blood.

It has been shown that the capacity of plasma to clot spontaneously increases *pari passu* with the removal of prothrombin. All the known facts point to the conclusion that the activity of prothrombin is necessary for spontaneous clotting. It thus appears that the problem of the maintenance of the fluidity of circulating blood is essentially that of the stabilisation of prothrombin.

Antithrombokinase and Antiprothrombin (Heparin)

Attempts have been made to explain the inactivity of prothrombin *in vivo* by postulating the presence of specific inhibitory substances in plasma. Collingwood and Mac-

precipitation with acetone and re-solution with 0·5 per cent. NaCl; the precipitate is layered over with equal parts of acetone and water to remove the NaCl. The precipitate is dissolved in water and evaporated to dryness. The final product is readily soluble in water, is stable at 100° C., and is an extremely active anti-coagulant *in vivo* and *in vitro*. An anti-coagulant resembling heparin is obtainable in minute quantities, when the blood plasma of fasting dogs is similarly treated. The precise composition of heparin is not yet known. In a recent letter, Prof. Howell informs me that he believes that it is a glycuronate and that besides preventing blood clotting it preserves blood platelets in a normal condition for twenty-four hours.

There is another reason for suspending judgment on the significance of heparin in blood coagulation. In two or three hours heparin loses its anti-coagulant action *in vivo* and produces increased coagulability of the blood. The following experiment illustrates these changes. Reed (1925) found that blood shed six minutes after the intravascular injection of 100 mg. of heparin into a 14-kilo dog remained fluid permanently. Serial bleedings during 1½ hours showed, however, a gradual restoration of coagulability. Blood shed an hour later clotted in 1 minute, 40 seconds, whilst that obtained after 40 more minutes had passed clotted in 40 seconds. It is difficult to reconcile these observations with the supposition that heparin permanently preserves the fluidity of circulating blood.

Many years ago Conradi (1901) found that the advanced autolysis of animal tissues produces substances that hinder the clotting of blood. Anti-coagulants can also be obtained by the autolysis of vegetable products, and one of these bodies possesses similar properties to heparin (Pickering and Hewitt, 1922, 1). It is possible that when the autolysis of cells and tissues is very active, sufficient heparin may be liberated into the blood-stream to impair the coagulability of the blood. It is significant that indications of the presence of heparin in blood have been obtained only in the plasmas of animals which are either fasting or are suffering from "peptone" shock. In both these conditions the occurrence of abnormal autolysis seems certain. A similar explanation seems applicable to the slightly decreased coagulability of

the blood in fevers, since in such conditions an excessive destruction of tissues occurs.

The Conclusions of Bordet

Thrombin is formed more rapidly when an oxalated solution containing prothrombin has been mixed with cytozyme for one or two hours and is then recalcified than when recalcification takes place immediately after the addition of cytozyme. Thrombin is formed less rapidly in freshly prepared oxalated plasma after the addition of cytozyme and dilute $CaCl_2$ than after the addition of cytozyme to serum. To explain these facts, Bordet and Delange (1913, 1914) suggest that the prothrombin of plasma (re-named "proserozyme") cannot directly unite with cytozyme, but is the mother substance of a body (serozyme) which appears in shed blood and unites with cytozyme, forming thrombin. In a later paper, Bordet (1920) reverts to the earlier suggestion that the plasma is stabilised by specific anti-coagulants, but points out that the material called proserozyme may be actually prothrombin masked by a protective colloid, which prevents, or at least retards, the reaction with cytozyme.

Stabilisation by Protective Colloids

It seems to me that the relationships of prothrombin in plasma provide an adequate explanation of its inactivity *in vivo*. Some of the prothrombin of plasma is loosely attached to fibrinogen, since it is readily removed by adsorption. A considerable amount is, however, more firmly bound by fibrinogen. It can be released only by drastic action, such as coagulating the fibrinogen to which it is united, or by extraction with chloroform. In the latter case thrombin is rapidly formed. For example, sufficient thrombin is obtainable by shaking with chloroform an oxalated plasma which has been rendered stable at room temperatures after recalcification (by the partial adsorption of prothrombin) to clot a larger amount of fibrinogen than exists in such plasma. It thus appears that much of the prothrombin is inactive, owing to the firmness of its union with fibrinogen. An explanation is required, however, of the stability of loosely bound prothrombin in unshed blood.

The general review of plasma in Chapter II. points to the conclusion that the fractions of protein obtainable from plasma are products of the dissolution of a single complex, and that this complex is held together either by adsorption or by secondary bindings which do not involve true chemical reactions. Similar unions of colloids possessing different stabilities are the distinctive feature in colloidal protection against the action of precipitant ions (Billitzer, 1905; Zsigmondy, 1920). It is established that the action of calcium ions is essential for the activity of prothrombin (Arthus and Pagès, 1890; Sabbatani, 1901). If plasma colloids are united in the manner suggested, the union of prothrombin with the more stable colloids of plasma provides sufficient explanation of the suppression of such action and of the stability of prothrombin in undisturbed plasma. In short, an inter-relationship of plasma colloids in which the more stable complexes are united to and protect the less stable complexes is the essential factor in the preservation of the fluidity of blood *in vivo* (Pickering, 1925, 1926).

The recent work of Reiner (1927) on the fractionation of euglobulin points in the same direction. Its chief constituent is a protein practically insoluble in water and in physiological saline, and this body is associated with a protective colloid. By dialysis, the union with the protective colloid dissociates and the euglobulin precipitates. After a first precipitation, the precipitate still contains considerable amounts of protective colloid and may be dispersed as a cloudy solution in saline. After repeated precipitations, the content of protective colloid and the dispersibility of the globulin decrease. It was also noted that the protective colloid protects the globulin from the denaturating action of ether-alcohol.

The activity of prothrombin in blood shed upon surfaces which it wets is also intelligible. The forces of contact-action dissociate plasma complexes, and protective unions no longer restrain the action of calcium ions on prothrombin. Many other changes occurring in blood plasma are explicable when viewed from this standpoint. A few examples may now be recalled:—

(1) Circulating blood and blood shed on paraffin-wax or on oil is not in contact with a surface which it wets. The normal

inter-relationships of plasma proteins remain undisturbed, with the consequence that fluidity is preserved.

(2) Any extensive damage to the vascular endothelium produces conditions similar to those occurring when blood is shed in a wound. The damaged lining of the blood vessels is wetted by plasma and by contact-action dissociates plasma complexes, and so releases sufficient prothrombin from bondage to initiate clotting. Thus it is that clots are formed.

(3) The addition of viscous substances to blood immediately after it is shed hinders clotting, but does not suppress coagulation when thrombin is formed (Pickering and Taylor, 1924). Similarly, blood shed immediately after the intravascular injection of a large amount of gum may remain temporarily fluid (Foster and Whipple, 1921, 3). Restraint of the movements of calcium ions by the viscous material, with a consequent delay in the change from prothrombin to thrombin, affords a sufficient explanation of these facts

(4) The addition of a small quantity of distilled water to the relatively stable plasma of the terrapin provokes clotting, whilst the addition of a like amount of isotonic saline has no such effect (Howell, 1914). The distilled water alters the osmotic pressures in the plasma and thus favours dissociation; the isotonic fluid leaves the osmotic pressures unchanged and does not promote disintegration.

(5) Both oxalated and citrated plasmas clot more slowly on recalcification than plasma which has not been so treated. The formation of unions of the salt and plasma, which are more stable than those of pure plasma, accounts for the delayed reaction. A longer time is required for the dissociation of these complexes than suffices for the dissolution of normal plasma.

(6) In the complete suppression of clotting by the addition of moderate amounts of neutral salts to blood, the unions of salt and plasma are more stable; the plasma is sufficiently stabilised to resist dissociation by contact action and its prothrombin remains inert. This conclusion is consistent with absence of thrombin in such plasmas, as observed by Wooldridge (1893). Similarly, the anti-coagulant action of heparin, of neutralised thymus nucleic acid, of "peptone" *in vitro* and partly, if not wholly, *in vivo*, may be ascribed to more or less firm unions of these bodies with plasma protein and consequent restraint of dissociation. A similar reaction explains the anti-coagulant action of several arsenobenzols, since these bodies also restrain the inception of clotting in which prothrombin suffers change, but do not suppress coagulation by thrombin.

The failure of stabilarsan (arsenobenzol combined with glucose) to suppress blood clotting (Anwyl-Davies and Mellanby, 1923) is, however, significant. This compound has no restraining action

on hæmolysis, whilst anti-coagulant arsenobenzols are also anti-hæmolytic (Pickering and Taylor, 1924) Attention has already been directed to the parallelism of behaviour in substances that suppress the inception of blood clotting and those that inhibit hæmolysis. It now appears that when certain affinities of arsenobenzol are satisfied by combination with glucose, the power to inhibit both these processes is lost. It seems apparent that similar reactions occur in the stabilisation of plasma and its corpuscles. In this connection it may be mentioned that "peptone," cocaine, neutralised nucleic acid and heparin each inhibits the lysis of blood platelets and the inauguration of blood clotting.

It should, however, be remembered that it is not yet established that the complexes of plasma are bound together by physical forces. The unions may be solely due to chemical action. If this be true, the stability of plasma *in vivo* may be ascribed to the satisfaction of the affinities of prothrombin by combination with fibrinogen and the instability *in vitro* to the dissolution of this combine by contact catalysis. It would then appear that there are two distinct modes of combination of fibrinogen and prothrombin—a stable and unstable form—and that the unstable combination participates in the inception of clotting.

The Hypothesis of Mills

A somewhat similar explanation of the inactivity of prothrombin *in vivo* has recently been published by Mills (1926, 2). He believes that thrombin cannot be produced from prothrombin so long as blood fibrinogen is unchanged. There is, however, no evidence that fibrinogen changes its nature directly the blood is shed, but evidence has been cited that it is rapidly dissociated from other plasma complexes. In a note added to this paper, Mills suggests that the dissociation of plasma constituents is the primary cause of clotting, but he assigns the stability of unshed plasma to chemical combinations. It is implied that fibrinogen is united to prothrombin chemically. This seems dissonant with the fact that a fraction of prothrombin can be separated from plasma by adsorption.

CHAPTER VI

Thrombins and Antithrombins

Thrombins—Description and occurrence—Methods of preparation—Properties—Composition—*Rôle* in blood coagulation—Mode of action—Inactivation in serum.

Antithrombins—Methods of preparation—Supposed presence in plasma—The antithrombic action of serum—Mode of action in preventing clotting.

Clotted blood hastens the coagulation of blood that is still fluid. It clots solutions containing fibrinogen as well as oxalated and citrated plasmas. The last two reactions are distinctive of a coagulant that is present in blood clots and in serum. This material is commonly called "thrombin," but has also been named "holozym" (Fuld and Spiro, 1904) and "thrombase" (Anwyl-Davies and Mellanby, 1923). A satisfactory definition of thrombin is not yet possible. A pure product has not been isolated. Moreover, important chemical differences exist in the thrombins prepared by different methods, and it is not known whether these differences are due solely to different degrees of purity or if bodies of different composition possess the properties of thrombin.

All attempts to obtain thrombin from blood which does not contain clots have failed. It is true that traces of thrombin can sometimes be detected in oxalated plasma. These are probably derived from clots in the cannula through which the blood is shed, since thrombin is not present in oxalated blood which has been shed through a freshly paraffined cannula. Thrombins are apparently produced by the changes that culminate in blood coagulation.

Several writers imply that thrombin cannot exist in a free condition in circulating blood. It is said to be neutralised by antithrombin (Mellanby, 1909,1; Howell, 1911; Mills and Mathews, 1924). These conjectures are opposed by a fact disclosed in the following experiment :—

Twenty c.cm. of thrombin (prepared by Howell's (1910) method), dissolved in 0·9 per cent. NaCl, were injected intravenously into an anæsthetised cat. Blood was shed through a paraffined cannula into sufficient sodium oxalate to suppress the clotting of an equal volume of normal blood. It coagulated at room temperatures, 16°–18° C., in ten to fifteen minutes, thus indicating that the injected thrombin had not been neutralised by antithrombin in the blood-stream.

The venoms of certain snakes (*Echis carinatus* and *Notechis sentatus*, *Crotulus terrificus* and *Lanchesis lanceolatus*) clot oxalated or citrated plasmas and thus behave like thrombin (Lamb, 1903; Martin, 1905; Barratt, 1920; Collins, 1927).

Mellanby (1909, 2) maintains that the coagulants of venom are pure kinase. To account for their clotting oxalated plasma, he suggests that sufficient $CaCl_2$ is present in the plasma for the activation of prothrombin by kinase Unlike purified thrombin, the venoms may either produce intravascular clotting or suppress the coagulability of circulating blood. They thus behave like a mixture of thrombin and tissue extract.

The Methods of Preparation and Properties of Thrombin

The procedure of Schmidt has already been mentioned (p. 3). It gives a thrombin that contains hardly any protein (Rettger, 1909; Mellanby, 1909, 1). This body is not completely destroyed by heating to 100° C., and is said to combine in definite proportions with fibrinogen and so produce fibrin (Rettger, 1909). It contains phosphorus and reacts much more readily with Hammarsten's fibrinogen, which contains cytozyme and prothrombin, than with fibrinogen that has been almost deprived of these bodies by adsorption with animal charcoal. It seems probable that the principal ingredient of this body is a cytozyme, but traces of thrombin may also be present.

The extraction of fibrin with an 8 per cent. solution of NaCl yields a thrombin associated with globulin. This material coagulates at 56°–58° C., and loses its activity (Gamgee, 1879). It contains phosphorus, and clots Hammarsten's fibrinogen rather more rapidly than that purified by adsorption. Its loss of activity at 56°–58° C. is probably due to the entanglement of the coagulant in the coagulum and its removal from solution.

The thrombin of Morawitz (1904) is obtained by mixing tissue extract and dilute $CaCl_2$ with serum; that of Bordet and Delange (1912) by incubating the washed *débris* of blood platelets with serum and dilute $CaCl_2$. These bodies are rich in both protein and phosphorus. They rapidly clot both Hammarsten's fibrinogen and that purified by adsorption. After heating to 60° C., they slowly clot crude fibrinogen (*i.e.*, in 6 to 12 hours), but do not clot fibrinogen at room temperatures when it has been purified by adsorption.

A very active thrombin was prepared by Mellanby (1909, 1), by treating the fibrinogen-prothrombin complex of bird's plasma with testis extract and dilute $CaCl_2$. It is freed from globulin by dialysis against water. This body gives the reactions of protein, contains phosphorus, is destroyed at 60° C. and, according to Mellanby, behaves like an enzyme. It clots Hammarsten's fibrinogen more rapidly than purified fibrinogen. A method of purification of this product has been described by Bleibtreu (1926), who does not regard it as an enzyme.

An even more active thrombin can be prepared by Howell's (1910) method. Washed fibrin is extracted with an 8 per cent. solution of NaCl for 48 hours in an ice-chest. The extract is filtered and repeatedy shaken with one-half its volume of chloroform, until the filtrate is neither precipitated by the addition of crystals of $(NH_4)_2SO_4$, nor coagulated by heating. Evaporation to dryness at 30°–40° C. gives a thrombin which retains its activity indefinitely. This body is soluble in water and in dilute saline solutions. It gives the biuret, xanthoproteic and tryptophane reactions. It does not react like an enzyme, and resists boiling for a short time. Its activity is enhanced by tryptic digestion and by putrefaction. It retains its activity when divested of phosphorus-containing material (Howell, 1925). It clots crude and purified fibrinogen at approximately the same speeds.

The extraction of serum with chloroform provides material containing thrombin (Minot, 1915; Dale and Walpole, 1916; Nolf, 1922). This extract contains both protein and phosphorus. It clots both intravascular and extravascular plasmas, including oxalated and citrated plasmas. When slowly introduced into

the blood-stream it suppresses the coagulability of the blood (Nolf, 1922, 2), thus behaving like tissue extracts containing protein-phospholipin complexes. On keeping, it loses its coagulant action *in vivo*, but retains its activity *in vitro*. On account of this reaction, it has been described as "nascent thrombin" (Mills and Mathews, 1924).

Serum contains protein-phosphatide complexes, closely resembling those of tissue extracts. It seems evident that this extract includes both thrombin and such bodies.

The addition of cephalin to fresh serum also gives material that clots both circulating blood and citrated plasma. It is an unstable product, since on keeping for half an hour its activity *in vivo* is lost and its power to clot extra-vascular plasma subsequently disappears (Mills, 1926, 2, 3). In two important qualities it differs from the thrombin formed *in vivo* when coagula are produced in the heart by the introduction of strings into its ventricles. The natural thrombin causes clotting only in the area adjacent to the strings, and produces hypercoagulability of the blood in the general circulation. It does not cause inhibited coagulability. In sharp contrast, serum treated with cephalin either produces extensive intravascular clotting, or, when minute quantities are injected into the blood-stream, suppresses coagulability. It thus behaves more like tissue extract than thrombin. Furthermore, cephalin, when injected intravenously, produces hypercoagulability of the blood (Hess, 1915, 2), and its addition to tissue extracts augments their coagulant action (Vines, 1921, 2). It seems certain that serum treated with cephalin contains either a compound or an adsorption-complex including cephalin, cytozyme and thrombin. It is unnecessary to assume, with Mills, that its activity *in vivo* is due to the formation of nascent thrombin.

Attention is re-directed to the dissociation of the thrombin prepared by Howell's method into two fractions (see p. 47). In reacting with impure fibrinogen, the water-soluble fraction gives typical fibrin, but the water-insoluble fraction yields a coagulum, resembling fibrin, that subsequently undergoes spontaneous lysis (Cekada, 1926). A similar lysis of blood clots sometimes occurs naturally, but it is not known if this is due to disassociated thrombin, or if un-

dissociated thrombin may react similarly. Further investigation of these bodies should yield important results in the problem of clotting and possibly explain the natural disappearance of small clots in the blood-stream.

The natural lysis of blood clots was ascribed by the earlier writers to bacterial action. It was shown, however, to occur under sterile conditions (Dastre, 1893). A specific lysin (*i.e.*, fibrinolysin) was postulated (Nolf, 1908). If Cekada's work is confirmed, this body may possibly be identified with thrombin.

The Composition of Thrombin

Current suggestions on the chemical constitution of thrombin are largely based on conjecture. According to Bordet (1920), thrombin is a complex containing calcium, protein and cephalin, the last-named body being derived from blood platelets. Howell (1925, 1) dissents from this conclusion, and believes that thrombin is prothrombin modified by the activity of calcium ions. The principal observations which have led to these divergent views have already been summarised (pp. 47, 54). Hekma (1924) describes both prothrombin and thrombin as agglutinins, and does not imply chemical differences in their constitution. In a recent review of blood coagulation, Mills (1926) suggests that thrombin is produced by a reversible chemical reaction in which prothrombin combines with cephalin and calcium. It is difficult to reconcile this conclusion with the preparation of a phosphorus-free thrombin by Howell (1925, 1), and by Cekada (1926). That fibrinogen can be clotted by more than one substance seems established by the previously-cited observation of Gratia on the clotting of oxalated plasma by staphylococci without the formation of thrombin. In the natural clotting of pure blood it is possible that more than one substance, or group of substances, may convert fibrinogen into fibrin and that both Bordet and Howell are correct in their descriptions of thrombin.

The rôle of Thrombin in Blood Coagulation

The majority of contemporary writers agree that the clotting of fibrinogen by thrombin is a fundamental reaction

in blood clotting. Several facts support this conclusion. In the so-called spontaneous coagulation of blood, thrombin is always formed. It promptly clots those natural fluids from which fibrinogen can be precipitated, such as the shed plasmas of birds, fishes and terrapins, most natural transudates, also artificial solutions of fibrinogen. There is then a *primâ facie* case that the spontaneous clotting of blood and the clotting of fibrinogen by thrombin are identical reactions.

Several investigators, however, have wholly or partly rejected this conclusion. Wooldridge (1886–1893) maintained that fibrin is formed by the chemical combination of two plasma colloids and that thrombin is a subsidiary reaction product. The fibrinogen known in the laboratory is said either to be absent in circulating blood, or to be present in insufficient quantities to participate materially in coagulation. The principal observations cited in support of these opinions are: (1) blood plasma which remains fluid after the intravenous injection of "peptone" is not clotted by thrombin, but may ultimately clot spontaneously, and (2) thrombin can be introduced into the blood-stream without causing intravascular clotting. The first of these statements is not always true, for Howell (1925, 1) has shown that the addition of large amounts of thrombin to "peptonised" blood provokes coagulation. Sufficient thrombin to clot all the blood of a cat, when it has been oxalated, can, however, be injected into the animal's circulation without producing clots. But there are difficulties in accepting the conclusion that fibrin is formed by the union of two plasma colloids without the intervention of thrombin. In inflammatory states an increase of the fibrinogen-prothrombin complex obtainable from plasma by salting-out runs parallel with the amount of fibrin formed by clotting, and both these increases may occur when there is no appreciable increase in the other fractions of protein obtainable from plasma. Coagulation by changes in fibrinogen-prothrombin complex proteins is thus indicated, and the thrombin theories of clotting appear justified.

Evidence has been recorded in this chapter which indicates

that thrombin is not neutralised in the blood-stream. An explanation of the inactivity of thrombin *in vivo* is therefore necessary. In the preceding chapters many facts have been described which show that the primary change in blood coagulation is the dissociation of plasma complexes. It again seems apparent that whilst fibrinogen is bound to other plasma constituents, it resists the action of thrombin, and that when it is dissociated from its normal relationships it is clotted by thrombin. The *rôle* of thrombin thus becomes intelligible, without postulating the many anticoagulants which have burdened the thrombin theories of blood coagulation with a constantly increasing load of assumptions.

The views of Nolf (1908–1927) are a development of those of Wooldridge. Thrombin is described as an unsaturated body formed from thrombogen during the union of three plasma colloids which are said to combine and form fibrin. The thrombin of shed blood is regarded as an unsaturated residue, and its coagulant activity is ascribed to this condition.

A somewhat similar conclusion is advocated by Wadsworth and the Maltaners (1927). They suggest that in shed blood ionised calcium, aided by contact catalysis, reacts with active lipoid, producing acid and a calcium-lipoid complex. Plasma protein, particularly fibrinogen, is altered by the acid and then combines with the calcium-lipoid complex, forming an insoluble product (fibrin). Thrombin is described as an intermediate product in this reaction and not as a body formed by the interaction of a definite fraction of plasma with protein-phospholipin complexes. These conclusions are based on the precipitation of acidified plasma by certain lipoids and on the finding that Bordet's preparation of prothrombin (proserozyme) contains fibrinogen. They ignore, however, several facts, such as the production of thrombin in solutions which have been deprived of fibrinogen, the extraction from plasma of a phosphorus-free product which behaves like thrombin and the formation of fibrin gels in the blood of the domestic fowl which is alkaline to litmus.

According to Mills (1926, 2), the clotting of fibrinogen by thrombin is of secondary importance in the coagulation of blood in wounds. The first step is said to be the clotting of fibrinogen by chemical combination with tissue juices.

In this process prothrombin is believed to be released from bondage and to be converted into thrombin which subsequently clots any fibrinogen remaining in the blood. It is difficult to reconcile these conclusions with the fact that blood shed through a paraffined cannula, without contact with tissue juices, on to a water-wettable surface, clots at 30°–40° C. as rapidly as blood in wounds. Tissue extracts undoubtedly participate in blood coagulation, but their action is probably concurrent with that of thrombin on fibrinogen.

The Mode of Action of Thrombin

A few contemporary investigators accept the suggestion tentatively made by Alex. Schmidt, that thrombin is an enzyme. Opposed to this opinion are the observations of Rettger (1909) and of Howell (1910) that thrombin combines in definite proportions with fibrinogen and so forms fibrin. The former observations are based on the action of a preparation which contains hardly any thrombin, but is rich in cytozyme. Howell's material, however, exhibits the typical reactions of thrombin, and is probably in a state approximating to purity. I am unaware of any observations that discredit his conclusion that it does not behave like an enzyme. Nevertheless, it is probable that a coagulant catalyst is sometimes associated with thrombin. Barratt (1920) found that the venom of *Echis carinatus* is active as a coagulant in concentrations of 1 in 3,000,000. He concluded that fibrinogen in solution is diphasic; also that thrombin modifies the phase-equilibrium by catalytic action and so produces clotting.

Other physical explanations have been suggested. Stuber and Sano (1922) maintain that thrombin acts by causing the swelling of fibrinogen. This conclusion is based on their finding that when thrombin and fibrinogen are dissolved in isotonic saline and separated by a semi-permeable membrane, clotting occurs after several hours, even if the thrombin is replaced by solutions of gelatin or starch. Partly denaturated thrombin is said to pass through the membrane and, by swelling, to abstract water from the fibrinogen and so produce

clots. These experiments were apparently made with a fibrinogen not completely deprived of prothrombin. The delayed clotting may be due to the slow conversion of prothrombin into thrombin, and not to the passage of thrombin through the membrane. This technique has also been criticised by Wöhlisch (1924). In reply, Stuber and Tanhauser (1924) deny the value of Wöhlisch's work. These experiments should be repeated with a fibrinogen deprived of prothrombin.

An entirely different interpretation is suggested by Hekma (1923, 1924, 1927). Thrombin is described as an agglutinin which provokes clotting by the aggregation, first of fibrinogen and then of fibrin. There are, however, several differences that distinguish thrombin from typical agglutinins. At the temperature of an ice-chest thrombin is almost inactive, whereas both iso-agglutinins and hetero-agglutinins are very active (Landsteiner and others, 1902, 1903; Jervell, 1921; Guthrie and Pessel, 1924). The agglutinative power of plasma and of serum is reduced between 36°–46° C. (Fåhraeus, 1921). Within this range of temperature the speed of clotting of pure cats' blood slightly increases, whilst that of human blood, obtained by finger puncture, remains almost constant (Pickering and de Souza, 1923). Agglutinins retain their activity in serum for several months (Hektoen, 1907): thrombin loses its activity in a few hours. Autohæmagglutinins (*i.e.*, the material which agglutinates an animal's own erythrocytes) are rarely found in serum. Thrombin is always present in fresh normal serum. The aggregation of particles of fibrin in the presence of thrombin may, perhaps, be more appropriately described as "pseudo-agglutination."

Although autohæmagglutinins are rarely present in blood, the capacity of erythrocytes to form rouleaux exists in almost, if not all, blood plasmas. Forces are thus developed which aggregate these bodies. The formation of rouleaux differs, however, from typical agglutination, in that it is favoured by moderate increases of temperature and the rouleaux are dispersed on cooling. The work of Northop and Freund (1924) and of Oliver and Barnard

(1924) points to the dependence of rouleaux formation on differences of potential between the corpuscles and the suspending medium, and it appears probable that the aggregation of particles in clotting blood may be partly due to the same cause.

Reviewing the facts as a whole, it seems probable that thrombin combines chemically with fibrinogen, and that either thrombin itself, or some body closely associated with thrombin, aids blood coagulation by a process resembling agglutination. When, however, the electrical charge carried by fibrinogen is altered, and in some cases after dilution, there is no evidence of agglutinative action. An apparently structureless gel is formed (Howell, 1916). But in the normal clotting of mammalian blood, the aggregation of fibrin particles into filaments and masses is always apparent when coagulation is observed under the ultramicroscope.

The Disappearance of Thrombin in Serum

Thrombin disappears in serum, but reappears on the addition of dilute acids and alkalis. The latter reaction was explained by Alex. Schmidt (1892) as due to the generation of thrombin from an inactive precursor present in serum. Morawitz (1903) also suggested that an inactive body is present in serum which is activated by acids and alkalis. Fuld (1904) described this body as a stable form of thrombin, which he named "metazyme." This material is now generally known as "metathrombin." Mellanby (1909, 1) maintained that metathrombin is an enzyme (a kinase), and that its production in serum by the addition of acids and alkalis depends on the splitting of the absorbed enzyme from serum protein. Landsberg (1913) reached the conclusion that thrombin is inactivated by adsorption to serum protein. This seems improbable from the work of Gasser (1916–17), who found that thrombin does not lose its activity when incubated with various proteins, or exposed to adsorbents. Several workers in Howell's laboratory suggest that metathrombin is a combination of thrombin and antithrombin (Weymouth, 1913; Gasser, 1916–17; Rich, 1917),

and until recently this conclusion has remained undisputed. From the rapid disappearance of thrombin in serum, after the addition of an anticoagulant protein obtained by the dissolution of tissue extract, and from the reappearance of thrombin by the subsequent addition of cephalin, Mills (1926, 1, 2), concludes that the inactivation of thrombin in serum is due to the regeneration of prothrombin by the removal of cephalin. In short, it is claimed that there is a reversal of the reaction by which thrombin is said to be produced. This conclusion is, however, inconsistent with the claims of Howell (1925, 1) and of Cekada (1926) that thrombin remains active after the removal of phosphorus-containing material. There is no doubt that the addition of crude cephalin to serum which has lost its coagulant activity restores its power of clotting fibrinogen, but experimentation with much purer products than those used in these investigations is necessary before a satisfactory decision can be reached.

Antithrombins

The maintenance of the fluidity of circulating blood by the interaction of coagulants and anticoagulants appears first to have been suggested by Spiro and Ellinger (1897), although implied by Alex. Schmidt (1872), who maintained that all cells contain both coagulants and anticoagulants. These suggestions, however, did not attract general attention until Morawitz (1903) deduced the presence of anticoagulant antibodies in plasma, from the difficulty he experienced in clotting blood, kept fluid by oxalates or fluorides, by the addition of either serum or thrombin. It is now known that the preparations used by Morawitz contain hardly any thrombin. The belief in the presence of antithrombin in the blood-stream thus arose from a misinterpretation of facts. At this period it was known that " peptone " has a greater anticoagulant action when introduced into the circulation than when added to blood shed into glass vessels (Schmidt-Mulheim, 1880). This was explained as due to the hepatic secretion of an excess of antithrombin, owing to the toxic action of the " peptone." The attention

of investigators was thus directed to the obtaining of antithrombin from the liver.

The Methods of Preparing Antithrombins

Doyon and his assistants (1910–1919) describe the following methods of preparing antithrombin :—

The liver of a dog is frozen and thawed three times during a period of forty-eight hours. During the intervals between the freezing it is exposed to the air at room temperature. It is then perfused with 0·9 per cent. NaCl, made alkaline by adding dilute sodium carbonate. The perfusate is not an anticoagulant, but when heated to 100° C. gives an antithrombin. Alternative methods involve the antoclaving of liver at 120° C., the exposing of minced liver to chloroform vapour and allowing the intestines to undergo autolysis. By these processes, antithrombins are obtainable from most of the organs of vertebrates, from the tissues of invertebrates and even from vegetable material (Pickering and Hewitt, 1922). It is, however, noteworthy that Doyon (1921) could not obtain any anticoagulant from the liver of normal rabbits, although Davis (1912) showed that large amounts of thrombin can be injected into the circulation of these animals without causing intravascular clotting.

The method used by Howell (1911-12, 1914) to demonstrate antithrombin in blood, is the heating of either oxalate or "peptone" plasma to 60° C. The filtrate contains material which neutralises thrombin.

An anticoagulant globulin, which possesses the power of inactivating thrombin, was obtained by Mills, Raap and Jackson (1921), by the extraction of dried lungs with benzene. It is the product of the dissolution of the protein-phospholipin complex of lung tissue and possesses no anticoagulant properties when it is combined with the phosphatide molecules. This material apparently differs from the antithrombin of Doyon, as it is destroyed at 100° C.

When physiological methods are employed, negative results are obtained. Menten (1920) found no anticoagulant in the liquid obtained by perfusing fresh livers with normal saline. Rettger (1909) obtained no evidence of the production of antithrombin after serial injections of thrombin into the blood-stream of dogs and rabbits. Attempts to produce

antithrombin by stimulating the liver with bile, bile salts, secretin and electrical stimuli have also given negative results (Denny and Minot, 1915). The extraction of the linings of blood vessels has, however, yielded contradictory results. Loeb (1904) could not find any anticoagulant. Hiruma (1923) extracted an antithrombin from each of the coats of the aorta. When fresh material is used, the results are different. Scrapings from the intima of the aorta contain a very active coagulant (Morse, 1919), whilst saline extracts of the aortic tissues of freshly-killed animals also hasten the clotting of blood (Mason, 1924). These experiments I have confirmed. An antithrombin can, however, be obtained when the linings of the aorta are incubated for 24 hours at 37° C., and then extracted with normal saline. The chemical evidence thus points to the production of antithrombin by either autolytic or *post-mortem* changes in cells or tissues, and not to its presence in a free condition either in liver, vascular endothelium, or blood-stream. In Chapter VIII. evidence will be cited indicating the possible production of antithrombin when the living tissues of mammals suffer abnormal autolysis. But there is no evidence of the formation of antithrombin and its passage into the blood-stream under normal conditions. Antithrombin is, however, normally present in the secretion of the buccal glands of the leech, in the heads of some parasites, and in the hepato-pancreas of the crayfish (Gratia, 1921, 2).

The Supposed Presence of Antithrombin in Bird's Plasma

Mellanby (1909, 1), found that 2 c.cm. of bird's fibrinogen dissolved in normal saline is clotted by 0·1 c.cm. of thrombin in 3 minutes, and that an equal amount of thrombin added to 2 c.cm. of bird's plasma required 5 minutes for coagulation. Since slight alkalinity favours the action of thrombin on fibrinogen, and as bird's plasma is distinctly alkaline, Mellanby interpreted his experiments as indicating the presence of free antithrombin in bird's plasma. If, however, plasma be regarded as a complex of proteins which resists the coagulant action of thrombin, these observations are

readily intelligible, without postulating the presence of free antithrombin in normal blood plasma. More time would be required for the dissociation of plasma complexes than is necessary for the action of thrombin on dissociated fibrinogen.

The Antithrombins of Serum

It has been amply demonstrated that antithrombic properties are developed in serum, but the mode of development indicates the formation of antithrombin by autolysis. Mellanby (1909, 1) compared the speeds of the clotting of standard solutions of fibrinogen by thrombin alone, and after incubation of thrombin at 30° C. with bird's serum. The incubated material required longer for the production of clots, the anticoagulant action being proportional to the duration of the incubation. Collingwood and MacMahon (1914) observed that an inhibitory power on thrombin is developed in mammalian serum by keeping it at room temperature, and becomes very marked after two days. This again points to autolytic processes. Serum, we have seen, contains protein-phospholipin complexes, and these, when broken up, yield an anticoagulant.

The Mode of Action of Antithrombins

Very little is known as to the mode of action of antithrombins. The anticoagulant of leech extract apparently acts upon the participant in blood clotting which is present in the greatest concentration. It not only neutralises thrombin, but also cytozyme. It apparently unites with these bodies in varying proportions, in the manner of adsorption compounds (Gratia, 1921, 2). The antithrombins prepared by Doyon's methods do not neutralise cytozyme. The statement of Mills (1926, 1, 2) that the antithrombin obtained by the disintegration of lung extract neutralises thrombin by the abstraction of cephalin and so regenerates prothrombin in serum, has already been mentioned. The action of this material on the phosphorus-free thrombin of Howell has apparently not been investigated.

According to Nolf (1926, 1927), the natural anticoagu-

lants of blood are protective colloids. He uses the term "antithrombosines" instead of "antithrombins." He believes that they are secreted in excess by the liver in response to conditions which tend to deprive blood of its fluidity. To establish the latter conclusion, it is necessary to show that during such conditions there are increases in more stable colloids of plasma.

In conclusion, it should be noted that although antithrombins neutralise thrombin in serum, the cumulative weight of evidence has convinced both Howell and Mills that a similar reaction is not the primary cause of the preservation of the fluidity of circulating blood (*vide* pp. 134–139). These investigators, therefore, have now reached the conclusion which has been promulgated from this laboratory during the last seven years.

CHAPTER VII

Fibrin

The properties of fibrin—The structure and modes of formation of fibrin aggregates—The estimation of the quantity of fibrinogen and of fibrin obtainable from blood plasma and other fluids—Variations in the amount of fibrinogen and fibrin obtainable from normal bloods—Factors which vary the amount of fibrin—Changes in the amount of fibrin during morbid states—The contraction of blood clots, with a special reference to their value in the diagnosis of disease.

FIBRIN separates from blood shed on rough surfaces as strings or as irregular masses, which are white, tough and elastic. It possesses the properties of coagulated protein. Its formation is hastened by mechanical disturbance, such as whipping with sticks. This has been explained as due to an anisotropic condition (Dieselhurst and Freundlich, 1916). The effect of disturbance is, however, modified by the number of platelets in the blood. When there is a marked shortage of these corpuscles whipping does not produce clots, but the fibrin may eventually be deposited in lumps (Burke and Tait, 1926). The forces of adhesion also modify the state in which fibrin remains. The gel formed when blood or plasma clots in a narrow glass tube does not contract until released from contact with the glass.

The first visible sign of clotting in shed blood is the formation of filaments on the surface exposed to the air. In frog's blood shed in the open air and in human blood shed in a moist chamber at room temperature, the filaments are formed one or two minutes before the whole of the blood clots. They rapidly dissolve in 0·9 per cent. NaCl, and so differ from typical fibrin, which only slowly dissolves, by autolysis, in dilute salt solution. Contact of the filaments with glass for one or two minutes converts them into material which possesses the solubilities of fibrin (Pickering

and Hewitt, 1921; Pickering, 1923). It seems probable that an intermediate product is formed which is converted into fibrin by contact action.

According to Hekma (1914, 1916, 1–7), fibrin exists in plasma as an alkaline hydrosol. He also states that alkaline solutions of re-dissolved fibrin may be made to gel again by appropriate treatment, such as the addition of dilute acids. This suggests that the gelation is a reversible process. It is not proven, however, that alkaline solutions of fibrin are identical with the precursor of fibrin in circulating blood.

The addition of dilute solutions of either HCl or NaOH to fibrin causes, first, swelling and, later, solution. The former occurrence is said to be due to the amphoteric nature of the amino-acids of fibrin, permitting the adsorption of both hydrogen and hydroxyl ions (Tolman and Bracebridge, 1919). The blood of animals of different species yields fibrin of slightly different qualities. For example, pig-fibrin dissolves more readily in dilute HCl than ox-fibrin (Fermi, 1896, confirmed by the present writer). Differences of solubility in dilute alkalis exist between the fibrin prepared from pure blood and that obtained from either oxalate or fluoride plasma. The former is insoluble in 0·02 per cent. NaOH, the latter is soluble (Barkan and Gaspar, 1923).

The Structure and Modes of Formation of Fibrin Aggregates

The resemblance of blood coagulation to crystallisation was suggested by Richardson (1858), but the first actual evidence of similarity in these processes is due to the work of Schimmelbusch (1885). He observed, in clotting blood, the formation of crystal-like needles which varied in length from 5 to 20 microns. Using dark-field illumination, Mayer (1907) found, when clotting is seen in an optically-clear field, that granules appear, which increase in size, coalesce into threads, and so form a network of fibrin. Similar appearances were seen by Cesana (1908) and also by Barratt (1920); the latter observer used solutions of fibrinogen, instead of blood, and employed the venom of *Echis carinatus* as a coagulant. The formation of fibrin by the deposition of bodies resembling crystals was re-discovered by Stübel (1914), was confirmed by Howell (1914), and is now generally

accepted. The needles are doubly refractile and, for this reason, are said by Stübel (1920) to be crystals. There are, however, several reasons for doubting this conclusion. Hekma (1916, 1–7) points out that the conditions which hasten the deposition of fibrin include those which assist both crystallisation and colloidal precipitation. Although the deposition of fibrin is favoured by mechanical factors, such as stirring or evaporation, the formation of fibrin-gels occurs in solutions which are far from saturation. Furthermore, the deposition of fibrin is favoured by high and not low temperatures and, by contact with rough surfaces, also the needles possess a certain flexibility and adhesiveness. Taking these facts into account, Hekma avoids the expressions "crystallisation" and "colloidal precipitation," and describes the formation of fibrin as occurring in two phases: Fibrin-alkali-hydrosol is changed into a simple hydrosol or is converted from a state of emulsion to one of true suspension; threads of fibrin are then formed by the apposition of these elements and from hitherto invisible complexes of fibrin molecules.

Blood clotting can take place without the formation of needles. When the oxalated plasma of the cat is diluted four times or more and then re-calcified, a structureless gel is produced. But the dilution of dog's blood, even a hundredfold, does not suppress the formation of needles (Howell, 1916). Important differences thus exist in the mode of fibrin-formation in the diluted plasmas of animals of different species. Differences in the precipitability of diluted fibrinogen by neutral salts have previously been noted (in Table II., p. 30). Species-variations in the stability of fibrinogen thus appear to be correlated with variations in the mode of formation of fibrin.

Other factors modify the structure of fibrin aggregates. A structureless gel is formed by the action of thrombin upon oxalated mammalian plasmas which have remained in a refrigerator for several days. Similar gels are produced by the action of dilute alkalis on fibrinogen and when negatively charged fibrinogen is acted upon by thrombin. Positively charged fibrinogen, when mixed with thrombin, gives, how-

ever, a flocculent precipitate, which either settles out or forms a membranous mass closely resembling the "membranous clots" which are formed at the onset of coagulation in slowly clotting bloods. From these facts, Howell (1916) concluded that in blood clotting the first stage of fibrin formation arises from the action of thrombin on fibrinogen particles which carry a positive charge, and that the later phenomenon of gelation is due to a slower reaction with fibrinogen bearing a negative charge. It has, however, been mentioned that Howell (1925, 1) found that thrombin can coagulate fibrinogen when the conditions preclude the union of oppositely charged particles.

The recent work of Hekma (1924) shows that serum produces two types of coagulation in both salted plasmas and in bird's plasma which has been deprived of thrombocytes. In the first type the ultramicroscope reveals the presence of sub-microns of fibrin, then the sudden formation of filaments composed of needles, followed by the growth of the needles and threads. In the second type flocculi of sub-microns are formed, which later agglutinate and become organised into groups of needles or into threads. The former process is described either as "pseudocrystallisation" or as "atypical agglutination"; the latter is compared with the behaviour of particles of vanadium pentoxide, that is to say, with that of an anisotropic substance. The initial flocculation in this mode of clotting may be due to the presence of an excess of cytozyme. It has been mentioned that the addition of cytozyme to plasma causes flocculation, and it is well known that some preparations of serum are rich in cytozyme and poor in thrombin.

Hekma (1924) also records that the thrombocyte-free plasma of the bird forms, when exposed to evaporation, a structureless gel which does not contain needles. Similar gels are formed when this plasma is shaken in a sealed tube for half an hour and may separate from human blood which exhibits an extreme shortage of platelets. In three cases of severe thrombocytopenic purpura (in which the platelets numbered respectively 15,300, 17,830, and 34,800 per c.mm.) the blood withdrawn by venepuncture gelled on glass and

dark-field illumination showed a structureless matrix in which only a few needles and filaments appeared in isolated areas. The addition of freshly prepared normal serum to these bloods produces a different ultramicroscopic picture. The deposition of fibrin commences as granules and needles and proceeds by the formation of threads, which interlace and form a network. It is evident that in both human and bird's blood the mode of fibrin formation is altered by the action of fresh serum, the presence of the latter material being correlated with production of quasi-crystalline accretions. It is noteworthy that the structureless gels are irretractile and that the needle-containing material contracts on ageing. Important physical differences thus distinguish these products, but it remains for future research to determine if they differ chemically.

According to Barratt (1920), fibrinogen in solution is diphasic, consisting of concentrated and dispersed fractions. By the action of thrombin (which is described as a catalyst), fibrin is formed out of fibrinogen in the concentrated phase. Subsequent increases in the thickness of the fibrin filaments arise by abstraction from the solution in a lower state of concentration, accompanied by disturbance of the equilibrium between the two phases of fibrinogen. These conclusions are based upon phenomena occurring when fibrinogen is clotted by snake venom, which may be entirely different from those of normal blood clotting.

Distinctive changes are also seen under the ultra-microscope when lung extract (which contains finely-divided particles) is added either to bird's blood or to citrated mammalian plasma, which has been deprived of most of its prothrombin by adsorption and then re-calcified. When the whole blood is used flocculi appear adjacent to the particulate matter, and this is followed by the lysis of thrombocytes. The flocculi rapidly increase in size and coalesce into gelatinous masses. Needles are deposited close to the disintegrating thrombocytes. They form threads. The gel thus assumes a quasi-crystalline structure. When the mammalian plasma is used, flocculi appear which rapidly grow into gelatinous masses and a few needles are formed in isolated areas. It is, however, clear that the clots are

almost wholly composed of material which has no resemblance to crystals. The changes revealed by the ultramicroscope are thus consistent with the conclusion mentioned in Chapter IV., that coagulation by tissue extracts differs from the clotting of pure blood.

The Estimation of the Quantity of Fibrinogen and of Fibrin obtainable from Blood Plasma and other Fluids

It is not possible in a work of this size to describe in detail the many methods used for the estimation of fibrinogen and fibrin. As summaries of technical processes are of no practical value, I shall only indicate and criticise the principles involved and supplement this information by a separate bibliography (*vide* Appendix C). Special attention is directed to the techniques of Whipple (1914), Cullen and Van Slyke (1920), Gram (1921), Leendertz and Gromelski (1922), Foster and Whipple (1921–1922), Ruszynák (1923); Zunz and La Barre (1925), and Chandler (1927).

Fibrinogen may be estimated by weighing the dried coagulum obtained from oxalated or citrated plasma by heating to 56°–60° C. In using this method, care should be taken to keep the hydrion concentration the same as that of the original plasma. If the views of Stüber and Sano (1923) are correct, that clotting is prevented in these plasmas by the formation of a highly ionised fibrinogen salt complex, an important source of error may exist. Such a complex may not be completely disassociated by heating to 56°–60° C. Fibrinogen is also estimated by weighing the dried precipitate obtained by appropriate salt-precipitation. When ammonium sulphate is used as the precipitant an often neglected source of error arises from the nearness of the salt-concentration at which the precipitation of fibrinogen ends and that of euglobulin commences. (See Table I., p. 6.) The use of sodium chloride is preferable, since fibrinogen is precipitated at nearly half saturation and euglobulin on saturation. If the fluid is diluted, as may occur in certain transudates, the results obtained by salt-precipitation should be checked by those given by heat-coagulation, as dilution may vary the amount of salt required to produce complete

precipitation. (Compare Tables I. and II.) Refractometric methods are also used for computing the amount of fibrinogen, particularly in small amounts of blood obtained by venepuncture. A source of error in these methods is the neglect of the difference in the amount of fibrinogen in arterial and venous blood.

Fibrin may be estimated by weighing the washed and dried material obtained by whipping a known quantity of blood. Care should be taken that the whipping is prolonged, as blood may clot in fractions at room temperatures. After the last fraction has been removed the residual fluid should be tested for fibrinogen by heating to 60° C. and by half saturation with sodium chloride. This method is unsuited to bloods exhibiting a shortage of platelets. In more recent techniques either oxalated or citrated plasma is used, and the fibrin formed on re-calcification is estimated either by weighing the dried and dehydrated material (Gram, 1921), or is computed from the difference in the nitrogen content of the plasma and serum. The latter method has been used in micro-estimations.

Strictly accurate results are unobtainable from mammalian plasmas, as the products of the lysis of blood platelets together with adsorbed prothrombin are present. The latter impurity can, however, be almost removed by repeated washing with acetone. The fibrin values of blood in various diseases should, therefore, be regarded as relative and not absolute figures. Appended to such data should be a platelet count of the bloods investigated, so that the amount of error can be judged. The conversion of fibrin into soluble products by fibrinolysis is said by Goodpasture (1912) to be also a source of error, but this can be prevented by the addition of a few drops of carbolic acid to the plasma (Perutz and Rosemann, 1925).

Variations in the amount of Fibrinogen and Fibrin obtainable from Normal Bloods

Estimated by the method of heat-coagulation, the amount of fibrinogen in normal human blood varies between 0·30 and 0·40 per cent. of plasma (Whipple, 1914). The figures

recorded by Frédericq (1878) for the horse are very similar (0·29–0·43 per cent.). By precipitating fluoride plasma with ammonium sulphate, Reye (1898) found 0·41 per cent. of fibrinogen in ox plasma, whilst by the same method 0·47 per cent. was obtained from rabbit's plasma (Müller, 1905).

Most of the recent work has been directed to estimating the amount of fibrin obtainable from normal and morbid bloods. The following table, abstracted from the recent paper of Foster (1924), shows the principal results obtained from the bloods of normal persons:

TABLE III.—*Fibrin Determinations in Normal Persons*

Figures in Milligrams per 100 c.cm.

		Men.		Women.		Men and Women.
		Fibrin per 100 c cm. of plasma.	Fibrin per 100 c.cm of blood	Fibrin per 100 c.cm. of plasma	Fibrin per 100 c cm. of blood	Fibrin per 100 c cm of plasma
Hammersten and Hedin (1914).	Maximum	—	—	—	650
	Minimum	—	—	—	101
	Average	—	—	—	420
Mathews (1920)	Maximum	—	—	—	600
	Minimum	—	—	—	150
	Average	—	—	—	375
Whipple (1914) (7 determinations of fibrinogen by heat coagulation).	Maximum	—	—	—	591
	Minimum	—	—	—	385
	Average	—	—	—	505
Foster and Whipple (4 determinations).	Maximum	364	178	—	—	—
	Minimum	316	163	—	—	—
	Average	335	172	—	—	—
Gram (1922) (50 determinations).	Maximum	360	190	380	210	—
	Minimum	200	110	210	120	—
	Average	270	130	290	170	—
Foster (1924) (42 determinations).	Maximum	446	210	470	233	—
	Minimum	256	112	255	143	—
	Average	332	163	344	179	—

The extremes of fibrin values are, however, rarely reached. In men the majority of results fall between the limits of 250 to 400 mg. per 100 c.cm. of plasma; in women, the common variations lie between 300 and 425 mg. per 100 c cm. of plasma (Foster, 1924). Similar results were obtained by Marakini and Yamaguchi (1924), when fibrinogen was estimated, by Wohlgemuth's method (1910), instead of fibrin. Many years ago Dastre (1893) found that arterial blood yields rather more fibrin than venous blood, and similar results have been recently obtained from the blood of dogs (Schultz, Nicholes and Schaefer, 1925). The amount of fibrin obtainable from the blood of normal individuals remains constant over considerable periods of time, both in man (Gram, 1921) and in the dog (Foster and Whipple, 1921, 2; Zunz, 1926). During normal pregnancy a great increase of fibrin values occurs (Levinski, 1903; Landsberg, 1910; Krosing, 1911; Gram, 1922). The average value for whole blood was found to be 94 mg. per 100 c.cm. higher in pregnant women (Foster, 1924).

Factors which vary the amount of Fibrin obtainable from Blood

Diets rich in protein increase the amount of fibrin obtainable from blood. Feeding on carbohydrates or on fats and fasting lead to a decrease, a lower but constant level being reached after three days of fasting. Plethora, induced by the transfusion of whole blood, first reduces the amount of fibrin obtainable, but a rapid return to normal occurs (Foster and Whipple, 1921, 2). Twenty-four hours after the loss of fibrinogen by hæmorrhage an increase occurs (Dreyer, 1893). In the dog the increase may lead to an excess of production, followed by a return to normality. All the evidence thus points to the constant production and utilisation of fibrinogen in the body (Foster and Whipple, 1921, 2, 3). The last-named observers did not find a similar replacement of other plasma proteins. Fibrinogen thus appears to be more actively engaged in metabolism than are the other protein fractions of plasma.

The amount of fibrin obtainable from blood is increased

by the introduction of heterologous serum, milk, peptone and gelatin into the vascular system (Moll, 1903; Löwy, 1916). The cause of these reactions is unknown, but stimulation of the production of fibrinogen appears more probable than the actual conversion of the introduced material into fibrinogen. Extensive injury to the epithelium of the intestinal tract also induces an over-production of fibrin (Foster and Whipple, 1921, 4), but it is not known if the fibrin formed is of the same composition as that produced by the spontaneous clotting of normal blood. A larger amount of fibrin is obtainable from blood confined in a vein isolated from the circulation than from circulating blood. It is suggested that fibrinogen is stored in the surrounding tissues and passes into the vein during stasis of the blood (Plass and Rourke, 1927).

Changes in the amount of Fibrin during Morbid States

An increase in the amount of fibrin obtainable from blood occurs in many diseases, particularly in those involving inflammatory processes, such as pneumonia, erysipelas, scarlet fever, rheumatism (Pfeiffer, 1897), peritonitis (Langstem and Mayer, 1903), syphilis (Winternitz, 1910), typhoid, pulmonary tuberculosis, pleurisy (Murakini and Yamaguchi, 1924), chronic nephritis, carcinoma, sarcoma and sepsis (McLester, Davidson and Frazier, 1925). Exceptionally high values have been found in pneumonia (Foster and Whipple, 1921, 2, 4), as much as 1,500 mg. per 100 c.cm. of plasma having been recorded (McLester and collaborators, 1925). Similar increases arise from experimental infection, notably with streptococci and pneumococci (Davide, 1925). Bacterial action is not, however, essential, since an over-production of fibrin arises from the presence of sterile abscesses (Foster and Whipple, 1921–1922, 4).

The salient feature of these observations is that processes which destroy tissues increase the amount of fibrin obtainable from blood, and this applies to liver tissue until its destruction is sufficient to impair the production of fibrinogen by that organ.

It is commonly assumed that the amount of fibrin obtainable from blood is an accurate gauge of the quantity of fibrinogen in plasma. This appears to be true in the case of normal blood (Howe, 1923), but may not apply to plasma contaminated by the products of tissue destruction. It is possible that the increase of fibrin found in acute inflammatory states may be due to additional clot formation by union of the disintegration-products of tissue with the fibrinogen of plasma. Lung extract is particularly active in this type of clotting, and it is significant that fibrin values are exceptionally high when extensive destruction of lung tissue occurs. Comparative estimations of the fibrinogen and fibrin obtainable during inflammatory states might yield interesting results.

Variations in the amount of fibrinogen in blood occur during both anaphylactic and "peptone" shock. These will be described and discussed later.

The Contraction of Blood Clots, with a special reference to their value in the Diagnosis of Disease

Most blood clots contract on ageing. This phenomenon is known as "syneresis." The contraction of clots is not confined to fibrin-gels; it sometimes occurs in the gels formed by the action of potassium hydroxide on chromium sulphate (Bancroft, 1921).

The chief interest of this subject lies in the fact that variations in the extent and speed of contraction of blood clots may be useful in the diagnosis of certain maladies. The loss of the contractility of clots in certain hæmorrhagic states will be described later and provides a means of recognising thrombocytopenic purpuras. Recent observations suggest that the study of the speed of clot-contraction may be valuable in estimating the rate of progress of certain diseases, particularly of hidden malignant tumours. Working with normal and diseased rabbits, Van Allen (1927) found that the speed of syneresis is greatest in healthy animals, but is reduced when vitality is impaired by various diseases. Decreases in the speed of clot-contraction occur early in the

inception of disease. For example, the onset of experimental rhinitis is indicated before the appearance of symptoms, and a malignant tumour, no larger than a pin-head, was associated with blood clots which contracted very slowly. Changes in the extent of clot-contraction do not apparently occur at the commencement of disease, but are well marked during the acute and terminal phases of various disorders. In transplanted epithelioma the curve of the growth of the tumour nearly coincides with the curve showing the decrease in the speed of clot-contraction during the progress of the disease. Evidence is produced which suggests that decreased speed of clot-contraction is correlated with the advance of the disease rather than with changes in nutrition. The cessation of the growth of a malignant tumour and the occurrence of metastasis appear to be readily recognisable by observing the speed of clot-contraction. Experiments should be conducted with the blood of carcinomatous persons.

The evidence bearing on the causation of syneresis appears contradictory. Some facts suggest that platelets are essential for that process; others imply that they are either not essential or merely hasten the contraction of fibrin aggregates.

Both re-calcified oxalate-plasma and hydrocele fluid give irretractile clots. When platelets are mixed with these fluids the contractility of the clots is restored in proportion to the number of platelets added. The addition of platelet extract does not, however, produce syneresis (Bordet and Delange, 1912; Opitz and Schober, 1923). In certain hæmorrhagic states thrombocytopenia is associated with the formation of irretractile clots (Duke, 1914; Stahl, 1920). Loss of contractility appears when the platelets fall below 75,000 per c.cm. of blood and is pronounced when only 45,000 per c.cm. are present (Opitz and Schober, 1923). Exceptions, however, occur (Rosenthal, 1928).

The destruction of platelets *in vivo* by anti-platelet serum produces blood which forms irretractile clots (Le Sourd and Pagniez, 1907–1913; Ledingham, 1914). Blood which has been oxalated and nearly deplateletised by filtration through a clay cell gives non-retractile clots, but such blood has lost part of its plasma protein.

Irretractile clots may be formed in pneumonia when the number of platelets is either normal or excessive (Hayem, 1896). Using injections of an extract of pneumococci in the rabbit, Van Allen

(1927) found that the resultant decrease in the speed of contraction of blood clots did not correspond with the amount of destruction of platelets and that the loss of contractility continued after the regeneration of the platelets. A temporary disappearance of platelets from the blood-stream may be produced without depriving the blood clots of their capacity to contract normally (Achard and Aynaud, 1908, 1 ; Pickering and Hewitt, 1923, 1924). The crystalline gels formed by the action of thrombin on fibrinogen contract rapidly, but structureless fibrin-gels are irretractile (Howell, 1916). The speed of the clotting of recalcified oxalated plasma determines the contractility of the clots formed. Contractile clots are produced at room temperature when the recalcification is rapid ; slow recalcification produces irretractile clots. At 40° C. both rapid and slow recalcification produce clots which contract rapidly (Pickering and Hewitt, 1924, 2). The citrated plasma of rabbits, which has been completely deprived of platelets, clots in 30–45 minutes on recalcification and gives apparently irretractile clots. When this plasma is mixed with concentrated serum and dilute $CaCl_2$ it forms clots which contract spontaneously (Roskam, 1926). Blood clots may not contract spontaneously when platelets are abundantly present in the plasma (Van Allen, 1927). The view of Howell (1916) that clot-contraction arises from the ageing and condensation of fibrin aggregates appears justified. It is, however, probable that the presence of platelets hastens this process in normal blood, possibly by modifying the adhesiveness and viscosity of the plasma.

CHAPTER VIII

The Suppression of the Coagulability of the Blood *in vivo*

Natural and acquired resistance against changes in the stability of the blood—The negative phase of blood clotting—The action of "peptone" on blood—"Peptone" tolerance—The anticoagulant action of arsenobenzols, of neutralised thymus nucleic acid, of oxalates and citrates; also of certain other substances.

The action of toxic substances on living organisms is modified by biological factors, as in natural or acquired resistance to certain toxins and in the increased response to intoxication by protein following sensitisation. It is also clear that the response to toxic stimuli varies in different animals, but these differences in behaviour are not yet explicable in either physical or chemical terms. Similar factors apparently control the resistance of circulating blood to disturbants of its stability. The intravascular injection of tissue extracts (prepared from testis, thymus, liver, lung, and other organs) into dogs produces profound changes in the stability of their blood, which may be manifested by partial intravascular clotting, and by the unclotted blood remaining either temporarily or permanently fluid when shed. The fluid blood is not only stabilised, but remains unclotted when additional amounts of tissue extract are introduced into the circulation. This condition may exist when the blood possesses the capacity to clot slowly when shed. But such blood is rapidly clotted *in vitro* by the addition of tissue extract. Part of the blood has thus acquired the power of resisting coagulation by tissue extract *in vivo*, but loses this quality when shed.

Extracts of testis or thymus produce intravascular clotting in pigmented rabbits, but do not deprive the blood that is left fluid of its capacity to clot *in vitro*. The circulating blood of albino rabbits resists the coagulant action of these extracts, and blood shed after their introduction into the circulation does not

exhibit inhibited coagulability (Halliburton and Brodie, 1895). Intravascular clotting and concomitant hypocoagulability of the blood can, however, be produced in both pigmented and albino rabbits by the intravenous injection of appropriate amounts of lung extract. The Arctic hare (*Lepus variabilis*) resists intravascular clotting by thymus extract during the winter when its fur is white, but is non-resistant during the summer when its fur is pigmented (Pickering, 1896).

Very variable results are obtained when appropriate amounts of " peptone " are injected into the blood-stream of fasting animals. In dogs almost complete suppression of the coagulability of the blood is usually produced, but some dogs are very resistant to this change, as was first observed by Gley (1896). Less marked inhibitions of coagulability occur in cats and rats. The resistance of the latter animals is correlated with their pigmentation, white rats being approximately twice as resistant as those with pigmented fur (Pickering and Hewitt, 1924, 1).

General metabolism, particularly the rate of consumption of nitrogenous material, modifies the response of blood plasma to influences which modify its stability. The differences in the coagulability of deplateletised blood which has been shed during the height of digestion and fasting have already been mentioned. The extirpation of the liver of dogs which have been fed on a meat diet and have been operated on during the height of digestion is rapidly followed by a decrease in coagulability of their blood, but this does not occur in dogs which have been fed on a diet poor in proteins (Nolf, 1905). Electrical stimulation of the depressor nerve does not appreciably alter the coagulability of the blood of normal rabbits, but may render the blood incoagulable when the animals have been starved for five days or longer. Some rabbits resist this change, even after longer periods of starvation. In the susceptible animals the urine becomes acid, indicating a metabolism like that of the dog ; in those which are not susceptible, the urine remains alkaline (de Waele, 1924). But extreme emaciation does not produce suppression of blood clotting in rabbits (Van Allen, 1927).

The full significance of these observations is not yet clear, but it seems established that the stability of blood plasma *in vivo* may be modified by biological activities similar to those which control the amount of resistance to toxins and by changes in the mode of autolysis of tissues.

There are three probable explanations of the action *in vivo* of substances which prevent the clotting of blood. They may remove from the blood-stream material which is essential for clotting. They may either act directly on the blood itself by forming complexes which are more stable than those of normal blood, or they may liberate anti-coagulants which pass into the blood-stream and restrain either the production or the activity of thrombin. Each of these explanations should be remembered in appraising the significance of the facts mentioned in this chapter.

The Negative Phase of Blood Coagulation

The suppression of the coagulability of the blood during poisoning by certain snake venoms has long been known (Fontana, 1787). The production of intravascular clots and of blood which remains fluid when shed, by the introduction of tissue extracts into the circulation, was first described by Wooldridge (1888) and were named respectively, the "positive and negative phases of blood coagulation." These reactions and the protection conferred against intravascular clotting by tissue extracts have been amply confirmed (Wright, 1892, and many other workers). The protection may, however, be only partial, as Mills (1921) observed that the too rapid injection of tissue extract into animals exhibiting the negative phase of coagulation may produce intravascular clotting. Martin (1893) demonstrated that the intravenous injection of a viperine venom (of *Pseudechis porphyriacus*) produces similar effects to those described by Wooldridge. He also noted that intravascular clotting occurs when small amounts of either venom or tissue extract are rapidly injected, and that intravascular clotting is not produced when similar or even larger quantities are injected very slowly. Blood shed after the

injection has been given slowly, remains either temporarily or permanently fluid. In the former condition it can usually be clotted by tissue extracts; in the latter it remains fluid after their addition. Other observers have shown that substances of widely different composition may provoke both the positive and negative phases of clotting. In this group are included certain synthetic colloids (Halliburton and Pickering, 1895), emulsions of tributyrine and of cream; also chloroform extracts of serum (Nolf, 1922).

Several explanations of these phenomena have been offered. Wooldridge (1888, 1893) found that blood plasma shed during the negative phase is precipitated by half saturation with NaCl, is coagulated by tissue extracts, by crude lecithin and sometimes by thrombin. From these facts, he concluded that the inhibition of clotting is not due to the exhaustion of coagulable material and suggested that it involves changes similar to those occurring in certain immunity reactions. In support of this suggestion, he stated that the intravascular injection of tissue extract may produce immunity against intoxication by anthrax. Certain facts point in the same direction. It has been mentioned that tissue extracts unite with fibrinogen and so form clots. The positive phase of coagulation is thus intelligible. These unions occur, in varying proportions, in a manner similar to those of toxins and antitoxins. The negative phase, except in cases where the whole of the fibrinogen is clotted and the blood so rendered incoagulable, is comparable to a number of *in vitro* reactions (described on p. 58), in which the mode of admixture determines the result produced. It should be recalled that larger amounts of the toxins of diphtheria and of ricin are neutralised by their respective antitoxins, when they are mixed in two fractions, with a time interval between, than when they are mixed at once (Dansyz, 1902). When kidney extract is added slowly, drop by drop, to shed bird's blood, a longer time elapses after the addition of the last drop and the occurrence of clotting than when the same amount of extract is added rapidly to the blood (Pickering and Hewitt, 1921).

Wright (1892) suggested that tissue extracts consist of two parts, one a nuclein which promotes clotting, the other a peptone-like substance which hinders clotting. Pekelharing (1895) claimed the finding of peptone in negative phase blood. But Martin (1893), and Halliburton and Brodie (1895), found no indication of its presence, and both the positive and negative phases of coagulation can be produced by the intravenous injection of substances which do not yield peptone on digestion with pepsin and dilute HCl (Halliburton and Pickering, 1895). The hepatic secretion of anticoagulants is improbable, since Arthus (1910) found that a negative phase can be produced when the liver is not supplied with blood.

Mellanby (1909, 2) states that the completely incoagulable bloods produced by the intravascular injection of viperine venoms do not contain fibrinogen and explains the different results arising from the action *in vivo* of both venoms and tissue extracts as follows: When the injection is rapidly given, fibrin is quickly formed and its molecules coalesce into filaments and so form clots. Slow injections produce a correspondingly slow formation of fibrin, which is removed from the blood-stream by the tissue cells before it can form filaments and produce clots. The latter process is said to occur to a less extent when negative phase blood retains its capacity to clot *in vitro*, and it is suggested that the quantities of fibrinogen and prothrombin left in the blood are so small that the generation of thrombin from prothrombin is sufficiently slow to permit its neutralisation by an antithrombin contained in the plasma. It has not, however, been demonstrated that antithrombin exists in a free condition in plasma.

Somewhat similar explanations have been offered by other writers. Nolf (1921, 1922) maintains that negative phase blood is defibrinated by the deposition of an ultramicroscopic film of fibrin on the vascular endothelium and that the fibrin is removed by a specific lysin. He also states that circulating blood can be completely defibrinated without the formation of visible clots This conclusion is based on the fact that negative phase blood which remains permanently fluid *in vitro* is not coagulated on heating to 56°–60° C., *i.e.*, at the temperature at which fibrinogen is coagulated. Furthermore, the addition of the appropriate amounts of neutral salts may not precipitate fibrinogen. But

these experiments do not prove that fibrinogen has been removed from the blood-stream. It is possible that the stability of the plasma has been so altered that it resists salt-precipitation and coagulation by heating to 50°–60° C. To establish Nolf's hypothesis it is necessary to show that proteins of plasma have been depleted to an extent equal to the amount of fibrinogen present before the production of the negative phase. Both Gutmann (1914) and Mills (1921, 2) found a shortage of fibrinogen in negative phase blood which ultimately clots. The former observer suggests that when intravascular clots are formed they adsorb fibrinogen from the plasma and so increase the deficiency of coagulable protein.

Very extensive intravascular clotting may possibly defibrinate the blood and so render it incoagulable, but "defibrination" does not explain temporary suppression of clotting. Negative phase bloods which remain fluid *in vitro* for half an hour may yield two-thirds of the normal amount of fibrin, and a greater shortage may occur in normal bloods without impairing their coagulability. In some negative phase bloods the yield of fibrin is, however, much less. Gutmann (1914) found that only one-ninth of the normal amount of fibrin and a quarter of the normal amount of thrombin may be obtainable. Very extensive intravascular clotting is produced by the intravenous injection of silica sols into rabbits, but the blood left fluid clots rapidly when shed. This occurs after the injections have been given either slowly or rapidly (Gye and Purdie, 1922). I have confirmed these observations and found that the fibrinogen and prothrombin can be reduced by this method to respectively one-fifteenth and one-tenth of their normal amounts, without impairing the capacity of the blood to clot rapidly when shed (in six to eight minutes at 16° C.). These results are inconsistent with the conclusion that a temporary negative phase arises from the removal of fibrinogen and prothrombin from the blood, but they are consistent with the suggestion that there are fundamental differences between negative phase blood and pure blood which has been partially defibrinated *in vivo*. The former appears to be stabilised; the latter has been shown to be hypercoagulable (Pickering and de Souza, 1923).

Other factors may contribute to the production of the positive and negative phases of clotting. In anæsthetised animals, asphyxia favours the formation of intravascular clots. The alkali reserve is reduced in negative phase blood (Mills, 1921) and in the "incoagulable" blood produced by intravenous injections of "peptone" (Underhill and Ringer, 1921; Blum and collaborators, 1924, 2; Baumberger, 1926). Both Schmidt (1892) and Deetjen (1909) found a considerable delay in the clotting of shed blood when the escape of CO_2 is prevented. Chio (1917) states that shed blood remains fluid when subjected to certain pressures of a mixture of CO_2 and O. Pickering and Hewitt (1921) suggest that tissue extracts, when injected into the circulation, react with fibrinogen and with the substances forming the alkali reserve. Hirsch (1924) maintains that the initial change in blood coagulation is a rapid alteration in the alkali reserve, whereby CO_2 is liberated. The recent work of Baumberger (1926) indicates that intimate relationships exist between the amount of the alkali reserve and the coagulability of the blood. He states that the optimum for clotting is represented by an amount of bicarbonates corresponding to about 30 volumes per cent. of CO_2. In both negative phase and "peptone" blood, the alkali reserve falls below this value.

The intravascular injection of tissue extracts causes a prompt fall in the number of circulating platelets. If the platelets are relatively abundant, whipping of the blood produces a small amount of fibrin. If the platelets are considerably reduced in number, no fibrin is obtained by whipping, but the blood eventually clots in lumps (Burke and Tait, 1926). Depletion of platelets undoubtedly contributes to the stability of negative phase blood, but it does not explain all the characteristics of the blood in that condition. Tissue extracts which have remained in suspension for one or two days lose their capacity of producing intravascular clotting, but retain the power of removing platelets from the circulation. The intravenous injection of the stale extracts produces some delay in the clotting of blood, but the delay is much less than that produced by fresh extracts. Furthermore, Burke and Tait (1926) demonstrated that there is no constant relationship between the number of circulating platelets and the amount of fibrinogen obtainable from negative phase blood.

The Action of "Peptone" on Blood

The observation of Schmidt-Mulheim (1880) that very large amounts of "peptone" are required for the suppression of blood clotting *in vitro* led to the belief that moderate

concentrations of " peptone " have no direct action on blood. Some of the earlier experiments were, however, opposed to this conclusion. Affanassiew (1884) found that blood shed from the artery of a dog into 1·225–1·5 per cent. of peptone remained fluid at 40° C. Pollitzer (1886) stated that a 2 per cent. solution of hetero-albumose inhibits the clotting of blood *in vitro*, and Halliburton (1888) noted that this substance delays the coagulation of salted plasmas. Shore (1890) observed that relatively large amounts of " peptone " do not suppress the clotting of lymph obtained from the thoracic duct and that smaller quantities inhibit clotting. In one experiment, in which the concentration of " peptone " was only 0·377 per cent., the lymph remained fluid for twenty-four hours (the pure lymph clotted in ten to fifteen minutes).

The action of " peptone " on shed blood was reinvestigated by Camus and Gley (1896), who found that eleven to fifteen times more " peptone " is required for the retardation of clotting *in vitro* than suffices when the " peptone " is injected intravenously. It was then assumed that the suppression of the coagulability of the blood which follows the intravascular injection of small quantities of " peptone " is due to the " peptone " stimulating the body cells to secrete an excess of antithrombin, which passes into the circulation and prevents blood clotting by neutralising thrombin. These opinions were reached without giving attention to the changes occurring in blood when it is shed upon a surface which it wets. That the difference in the amounts of " peptone " required for the suppression of the clotting of shed and circulating bloods is largely due to such changes is shown by the following observations:

The introduction of at least 0·3 per cent. of " peptone " into the circulating blood of the cat is required for the production of a marked inhibition of blood clotting. The addition of this concentration of " peptone " to cat's blood *in vitro* does not suppress clotting if the blood is shed without preventing the surface changes which initiate clotting. When, however, the surface conditions of the blood are partly preserved by bleeding through a paraffined cannula into a paraffined vessel, the results are different. In two typical experiments the presence of

0·3 per cent. of "peptone" respectively delayed the clotting of blood shed on paraffin for 80 and 109 minutes (Pickering and Hewitt, 1922, 1). This inhibitory effect is not produced if the blood has remained in contact with glass for two or three minutes before it is transferred to a paraffined vessel and mixed with "peptone." Under these conditions, eight to ten times more "peptone" is required to prevent clotting. The instantaneous mixing of 0·3 per cent. of "peptone" with blood shed in glass vessels has hardly any effect on the speed of blood-clotting, but higher concentrations (0·6–1 per cent.) distinctly retard clotting. When more stable bloods are used, the anticoagulant action is more marked. Nolf (1922, 1) found that 0·6 per cent of "peptone" suffices *in vitro* for the complete suppression of the clotting of the blood of the domestic fowl, whilst the addition of 0·4 per cent. delays for four hours the clotting of tortoise's blood (Pickering and Hewitt, 1924, 1). Bloods that resist the anticoagulant action of "peptone" *in vivo* also resist such action *in vitro*. For example, relatively large injections of "peptone" are required to decrease the coagulability of the blood of rats and a concentration of 0·3–0·4 per cent of "peptone" has hardly any anticoagulant action on rat's blood shed into paraffined vessels (Pickering and Hewitt, 1924, 1).

It thus appears that the condition of the blood determines its behaviour in the presence of "peptone" and that the anticoagulant action of "peptone" may be as active *in vitro* as *in vivo* when the surface conditions of the blood have not been greatly disturbed.

In a recent review of this work, Nolf (1926) admits the anticoagulant action of "peptone" on shed blood, but maintains it is fundamentally different from that produced *in vivo*. It is difficult to reconcile this conclusion with the following facts. Blood or plasma which has been "peptonised" *in vivo* exhibits the following characteristics: It is coagulated by the passage of a stream of CO_2, by acidification, by dilution with distilled water, by passage through a clay cell and by the addition of chemically inert powders (Fano, 1882; Wooldridge, 1884–1885, 1893; Gratia, 1921). It is not clotted by dilution with 0·9 per cent. NaCl (Howell, 1912). All these characteristics are exhibited by bloods and plasmas, which have been kept fluid by the addition of small amounts of "peptone" *in vitro*.

There is then a considerable amount of evidence which

indicates that the anticoagulant action of " peptone " is primarily due to its interaction with plasma. It seems established that " peptone " unites with plasma and forms a relatively stable complex which resists disintegration and coagulation.

A number of other facts are consistent with these conclusions. The first action of " peptone " *in vivo* is the production of hypercoagulability of the blood (Nolf, 1905, 1 , de Waele, 1921). The tension of CO_2 in the blood is decreased (Labousse, 1889 ; Salvioli, 1892 ; Baumberger, 1926). Slight flocculation and changes in the surface tension of the plasma are also produced (Zunz and Gyorgi, 1914 ; Kopaczewski, 1921 ; Gautrelet, cited by Hanzlik, 1924). These changes point to disturbance of plasma complexes prior to their union with peptone. " Peptone " hinders the lysis of blood platelets (Affanassiew, 1884) and restrains the hæmolysis of erythrocytes (Pickering and Taylor, 1924). A general stabilisation of the blood is thus indicated. " Peptone " plasma differs from plasma kept fluid by the addition of antithrombin (obtained by Doyon's method, described in Chapter VI.). The changes that produce thrombin are suppressed in " peptone plasma " (Gratia, 1921). In antithrombin plasma the formation of thrombin is not prevented, but the thrombin is inactivated. Hirudin prevents both the formation of thrombin and the coagulant action of thrombin on fibrinogen. But hirudinised plasma differs radically from " peptone " plasma. It is not clotted by CO_2, by dilution with water, nor by acidification (Gratia, 1921).

" Peptone " produces other effects *in vivo* which may augment its anticoagulant action. The temporary disappearance of platelets from the circulation during " peptone " shock has been mentioned, and accounts for much of the delay in blood clotting. In some carnivores, especially in dogs, the intravascular injection of " peptone " may possibly stimulate the autolysis of cells and tissues and so produce anticoagulants, which pass into the blood-stream. A considerable amount of evidence suggests, but does not prove, that the liver is the seat of such a reaction.

Contejean (1895) and Gley and Pachon (1895) respectively claimed that " peptone " injections have no anticoagulant action after ligature either of the abdominal arterial trunks or of the hepatic lymphatics. Starling (1895–1896) and Delezenne (1896)

could not, however, confirm these statements. Hédon and Delezenne (1896) established an Eck's fistula in two dogs and, as far as possible, removed their livers. In these animals the intravascular injection of "peptone" did not delay blood clotting. Delezenne (1896) reported four similar experiments, only one of which was described in detail. In the first two experiments several hours elapsed after the operation and before the "peptone" was injected. In the experiment described by Delezenne, five hours passed before the injection of "peptone." Similar results were obtained by Nolf (1905, 1, 3) after the total extirpation of the liver in anæsthetised and narcotised dogs. The common features of these experiments are impairment of vitality by prolonged and severe operations, also by narcosis, leading to a decrease of oxygen and an increase of CO_2 in the blood. Working with anæsthetised cats, Pickering and Hewitt (1922, 2) found, in both intact animals and in those deprived of hepatic activity, that the intravascular injection of "peptone" inhibits blood clotting, and that the anticoagulant action is annulled during partial asphyxia, when there is an excess of CO_2 in the blood. They also showed that the anticoagulant action is restored when the animals respire oxygen or are overventilated. The earlier results may thus be due to an increase of CO_2 in the blood and not to a loss of hepatic activity. In reviewing this work, Nolf (1926) admits the demonstration of the anticoagulant action of "peptone" in animals deprived of hepatic activity and leaves uncriticised the statements respecting the failure of "peptone" to inhibit clotting when the CO_2 in the blood is increased.

Doyon (1921) states that he has produced incoagulable blood by injecting "peptone" into dogs deprived of hepatic activity, but that this only occurs rarely. Nolf (1922) records as "a curious occurrence" that the anticoagulant action of "peptone" on the circulating blood of the domestic fowl is actually greater after extirpation of the liver. However, Nolf (1926) adheres to his conclusion that "peptone" stimulates the hepatic secretion of protective colloids (which he calls "antithrombosines"), and that these bodies assist in the suppression of blood clotting. He bases these conclusions on the following observations: Smaller quantities of "peptone" suppress clotting in intact animals than in those deprived of hepatic activity. The blood of dogs which has been mixed with "peptone" and has remained in the blood vessels of an excised liver, does not clot when shed (Nolf, 1905, 1). Injections of "peptone" which are insufficient to suppress the coagulability of circulating blood delay the clotting of lymph shed from the thoracic duct (Shore, 1890). The first of these observations may be due to the increase of CO_2 in the blood. The loss of coagulability of the blood after hepatic perfusion

may be due to the autolytic production of an anticoagulant by a moribund liver. It has been mentioned that freshly prepared tissues do not yield anticoagulants when extracted with normal saline and that stale tissues yield such bodies. It may be added that chemical or electrical stimulation of the livers of normal dogs does not produce an anticoagulant, neither does perfusion of the liver with normal saline (Menten, 1920). The third observation may be due to the greater stability of lymph.

Despite these possibilities, "peptone" may stimulate the production of anticoagulants by exciting abnormal autolysis, but only in animals possessing a very active nitrogenous metabolism. Nolf (1905, 1) observed that a mixture of rabbit's blood and "peptone" clots readily after remaining in the liver. It has been mentioned that protein-phospholipin complexes yield, on disintegration, an anticoagulant globulin. The production of incoagulable blood by the stimulation of the depressor nerve of starving rabbits should also be recalled. In these animals self-digestion may well produce anticoagulants in the liver, which may be carried into the circulation when hepatic secretion is stimulated. But this evidence lends no support to the still current conclusion that the normal livers of all mammals secrete antithrombins and so preserve the fluidity of circulating bloods.

Other explanations of the anticoagulant action of "peptone" have been offered. Dastre and Flouresco (1896), also Mellanby (1909, 2), suggest that it is solely due to the production of an excess of alkali by the liver. Gratia (1921) opposes this suggestion, because he was unable to find the slightest difference between the alkalinity of "peptone" and normal plasmas. Observations have, however, been cited in this chapter which indicate that changes in the relationships of the tension of CO_2 in the blood and the alkali reserve may contribute to the maintenance of the fluidity of the blood.

Both Popielski (1913) and Doyon (1919) recognise the difficulties in accepting the opinion that the anticoagulant action of "peptone" is due to the hepatic secretion of antithrombin. They suggest that antithrombin is secreted by the vascular endothelium and so passes into the blood. But despite many attempts, antithrombin has not been extracted from the freshly prepared tissues of blood vessels. Nolf (1911, 1922) maintains that the primary cause of the suppressed coagula-

bility of blood peptonised *in vivo* is defibrination of the plasma. This appears improbable. Foster and Whipple (1921) obtained more than 90 per cent. of the normal amount of fibrin from blood which had been rendered incoagulable by the injection of "peptone." Zunz (1926) states that the quantity of fibrinogen in such blood may be almost normal or may be increased or decreased, the increases varying from 2·3–10·7 per cent., the decreases from 0·84–37·44 per cent.

Mills (1926, 4) suggests that the action of "peptone" *in vivo* is "a typical immune reaction and may provide approach for study of immunity on chemical lines." He holds that the initial hypercoagulability of the blood following "peptone" injection is due to the union of plasma and "peptone," and that this provokes the outpouring of cephalin-absorbing proteins, which regenerate prothrombin from thrombin and so prevent clotting. It is too early to estimate the value of these suggestions, but they do not explain the anticoagulant action of "peptone" on blood shed into paraffined vessels, unless "peptone" disintegrates plasma and liberates anticoagulants.

"Peptone" Tolerance

It has long been known that dogs which have received an intravenous injection of "peptone" may exhibit complete resistance to subsequent injections. The tolerance continues after the blood has regained its coagulability, but is usually lost in twenty-four hours. It has also been demonstrated that the slow intravascular injection of "peptone" does not prevent blood clotting (Fano, 1881; Contejean, 1895; Gley and Leblas, 1897). The tolerance was explained by Fano (1881) as due to the exhaustion of an anticoagulant, which he believed to be liberated by the first injection; the disappearance of the tolerance was ascribed to a fresh production of anticoagulant. Contejean (1895, 1, 2) claimed the production of "peptone" immunity by using serum derived from peptonised blood as a vaccine, but he subsequently accepted Fano's hypothesis. Spiro

and Ellinger (1897) supported the theory of immunisation and suggested that body fluids, particularly lymph, contain a substance which neutralises anticoagulants. Nolf (1902) maintained that small amounts of "peptone" provoke an immunity reaction in the liver. He found that dogs which have been rendered tolerant to "peptone" remain in this condition when their blood has been replaced by that of normal dogs; also that normal dogs are not immune to "peptone" when they have been transfused with the blood of dogs that have acquired resistance to "peptone."

In later papers, Nolf (1921, 1922) suggests that the primary cause of the stability of the blood in "peptone" shock is defibrination of the plasma. Presumably, he explains the disappearance of tolerance by a regeneration of fibrinogen. Mellanby (1909, 2) concludes that the tolerance is due to the exhaustion of alkaline substances in the liver, but he does not offer any direct evidence in support of his opinion. Hepatic activity is not essential for the production of tolerance of "peptone." Pickering and Hewitt (1922, 2) showed that serial injections of small amounts of "peptone" into cats deprived of hepatic activity, produce tolerance to subsequent injections of amounts of "peptone," which are sufficient for the suppression of the clotting *in vitro* of the blood of normal cats for twenty-four hours. De Waele (1924) found fluctuations in the coagulability of the blood after the intravascular injection of substances which disturb the stability of circulating plasma, hypercoagulability being commonly followed by hypocoagulability. He suggested that any substance which increases the coagulability of the blood subsequently evokes the secretion of antithrombin. The first action of "peptone" is the production of hypercoagulability; the subsequent suppression of clotting is ascribed to the secretion of antithrombin. If this be true, tolerance is due to a temporary immunity to the second reaction. But, both silica sols and purified thrombins provoke hypercoagulability in circulating blood without producing either a subsequent suppression of blood clotting or any change resembling immune reactions. Furthermore, the hypocoagulability of the blood following

the intravascular injection of foreign material is often due to deplateletisation.

In certain characteristics, the production of tolerance to "peptone" differs from immunisation. A much longer period is required for the development of immunity to bacterial toxins than for the appearance of tolerance of "peptone." The duration of the former condition is much longer than that of the latter. The ratio $\frac{\text{albumin}}{\text{globulin}}$ in plasma is commonly increased during immunisation, but may remain unaltered or may be slightly decreased during "peptone" tolerance. The investigation of these differences should throw a considerable amount of light on the changes produced during immunisation and tolerance. In Chapter II. evidence is cited which shows that the plasma of adult animals contains all that is necessary for immune reactions; that just mentioned suggests that the changes which produce tolerance also arise in plasma. It appears that one of the functions of blood plasma is the suppression of toxic activities.

The Anticoagulant Action of Arsenobenzols, of Neutralised Thymus Nucleic Acid, of Oxalates and Citrates, also of certain other Substances

A knowledge of the toxic action of arsenobenzols on the blood is of practical importance, on account of the use of these substances in the treatment of venereal and other diseases. The anticoagulant action of arsphenamine and of its derivatives, novarsenobenzol and sulfarsenol, on both circulating and shed blood has been amply demonstrated (Tzanck, 1921; Flandin and Tzanck, 1921; Laurnoy, 1921). The anti-hæmolytic action of novarsenol (Billon) has already been mentioned. Stabilarsan (arsenobenzol combined with glucose) does not, however, alter the speed of blood clotting (Anwyl-Davies and Mellanby, 1923) and does not suppress hæmolysis (Pickering and Taylor, 1924). The toxicity of alkaline arsphenamine solutions is reduced by the addition of certain hydrophilic colloids, especially by gelatin. Much

larger doses are tolerated, and the intravenous injection of amounts which, without the protective colloid, produce severe reactions, may have no effect on the stability of the blood (Oliver and others, 1923).

A few other facts are known. Plasma which has been rendered incoagulable by the intravenous injection of novarsenobenzol is clotted *in vitro* by the addition of either serum or $CaCl_2$ (Laurnoy, 1921; van Dyke, 1925). Opalescence has been observed in plasmas which contain sufficient arsphenamine to remain fluid when shed (Oliver and Douglas, 1923), and precipitates may be formed after its addition to plasma and serum *in vitro;* also in pulmonary blood after the injection of arsphenamine hydrochloride (Smith, 1920). Alkaline solutions of arsphenamine produce, however, less precipitation and this may be due to the dispersing effect of the excess of alkali of the disodium salt (Voegtlin, 1925). Concentrations of novarsenol (Billon) and of sulfarsenol which are sufficient to render shed blood incoagulable do not prevent the clotting of fibrinogen by thrombin. It has also been demonstrated that much smaller amounts of novarsenobenzol (neosalvarsan) suffice for the suppression of clotting when injected intravenously than when added to shed blood (Nolf, 1922, 1).

It is clear then that all these medicaments, except stabilarsan, may profoundly alter the stability of the blood. The mishaps which follow overdosage or too rapid intravenous injection are thus intelligible. Emile-Weil (1924) believes, however, that the toxic action of arsenobenzols is not due to their anticoagulant action. This conclusion may be correct, since an equal or even greater inhibition of blood clotting is produced by heparin, without any signs of intoxication. But the importance of the blood in the physiological response to arsenobenzols is illustrated by the following observations. Arsphenamine agglutinates erythrocytes *in vitro*, and the intravenous injection of moderate amounts may produce multiple emboli in the lungs and other organs (Karsner and Hanzlik, 1920; Oliver and collaborators, 1922, 1923). Less toxic action follows the injection of blood mixed with a moderate amount of

arsenobenzol than that produced when an equal amount of this arsenical compound is injected alone (Flandin and Tzanck, 1924). Lethal action is recorded by Rosenbloom (1924) as following the administration of a therapeutic dose of arsphenamine to a hæmophilic who was also syphilitic, and it will be shown later that the essential defect in hæmophilia is an abnormal stability of the blood.

Syphilitic cases have been recorded in which arsenobenzols produce exceptionally severe reactions. In certain forms of syphilis the condition of the blood so closely resembles that of hæmophilia that they have been described as "acquired hæmophilia" (see p. 189). It is possible that these facts may be related and that "accidents" in the careful use of arsenobenzols may be so explained. Using a simile, it appears probable that the plasma acts like a "shock-absorber" when exposed to moderate amounts of arsenobenzols, and that when its stability is considerably increased it no longer behaves in this manner. If subsequent observations justify this suggestion, observations on the stability of the blood, as indicated by its coagulability when shed, should reveal hypersensitivity to arsenobenzols.

Several explanations have been offered of the anticoagulant action of arsenobenzols. Laurnoy (1921) suggests that there is a reaction in plasma with thrombin or its precursors. Helmonowa (1922) states that the changes which normally produce thrombin are suppressed. A similar conclusion was reached by Pickering and Taylor (1924). Oliver and Douglas (1923) suggest, however, that the reaction is with fibrinogen. Nolf (1922, 2) maintains that the anticoagulant action *in vivo* is fundamentally different from that *in vitro*. In the former case the plasma is defibrinated without the formation of visible clots. In the latter, the stability of the plasma is increased by its union with the medicament. But the loss of coagulability after the intravenous injection of an arsenobenzol may be only temporary. In such cases stabilisation is indicated, not defibrination.

Anwyl-Davies and Mellanby (1923) have provided indirect evidence of the union of arsenobenzols (except stabilarsan) with fibrinogen, and the work of van Dyke (1925) strongly

supports this conclusion. It also suggests the occurrence of accessory inhibitory action on thrombin, which appears to be reversible.

In the preceding chapters much evidence has been cited which suggests that in plasma fibrinogen and prothrombin are bound together into a complex which behaves as a co-ordinated whole. The stabilisation of fibrinogen thus implies the maintenance of prothrombin in a stable condition, and it is generally recognised that changes in prothrombin are essential for the inception of blood clotting. The conclusion that arsenobenzols unite with fibrinogen, forming a stable combination, is, therefore, consistent with evidence that they suppress the inception of blood clotting. When however, non-toxic doses of arsenobenzols are given the coagulability of the blood is unaltered, or only slightly altered, and the reactions just described either do not occur or are reversed by dilution.

In acid solutions appropriate concentrations of arsphenamine and of certain of its derivatives, such as salvarsan, coagulate and precipitate plasma proteins *in vitro* (Michaelis, 1910; Fleig, 1914, and several other observers), and a like reaction *in vivo* follows the intravascular injection of lethal amounts of arsphenamine into rabbits and rats, the coagula and precipitates being most marked in the blood vessels of the lungs (Lucke and Kolmer, 1921). These reactions provide an explanation of the fatalities which have followed the intravenous injection of unneutralised arsphenamine, which has been insufficiently diluted. Neoarsphenamine, sulpharsphenamine and tryparsamide, as at present manufactured, are said not to coagulate the proteins of serum *in vitro*, and there is no reason to assume that they coagulate or precipitate circulating plasma (Kolmer, 1926). Alkaline solutions of arsphenamine, in concentrations up to 10 per cent., do not precipitate or coagulate blood, but precipitation of the arsphenamine base may occur together with agglutination of erythrocytes. These facts are of considerable importance in the therapeutic use of arsenobenzols, and have been reviewed, with a bibliography, by Kolmer (1926).

Doyon (1921) showed that neutralised thymus nucleic acid suppresses the coagulability of both circulating and shed blood; also that smaller amounts of this anticoagulant are required for the former than for the latter reaction. He

explains the inhibition of the clotting of shed blood as due to direct action on the plasma, but suggests that the greater restraint of clotting *in vivo* arises from the secretion of an antithrombic nucleoprotein, either by the liver or by the vascular endothelium. The increase of anticoagulant action *in vivo* can, however, be explained without this assumption. The intravascular injection of neutralised thymus nucleic acid removes blood platelets from the circulation and so delays the inception of clotting. Blood, when shed and then mixed with this anticoagulant, contains a normal number of platelets and consequently clots more rapidly. Larger amounts of neutralised thymus nucleic acid are required for the prevention of the clotting of blood which has remained for a few minutes in contact with glass, but is still completely fluid, than when the mixing is made immediately after the blood is shed. There is actually a progressive decrease in anticoagulant action which corresponds with the duration of the contact of the blood with the glass (Pickering and Taylor, 1924). It is evident that this anticoagulant inhibits the pre-clot changes in blood and that the reaction is impaired by contact-action. It may be added that this suppression of clotting is as marked in animals deprived of hepatic activity as in those in which the liver is active (Pickering and Hewitt, 1924).

Considerable difficulty has been experienced in testing the anticoagulant action of soluble oxalates and citrates on circulating blood, owing to the toxicity of these salts. Mills (1921) found that the blood of a rabbit which had been completely prostrated by the injection of potassium oxalate exhibited a normal speed of clotting. The suppression of blood clotting in frogs, dogs and rabbits by the intravascular injection of sodium citrate was, however, demonstrated by Sabbatani (1900–1901). Pithed cats exhibit greater resistance to the toxic action of potassium oxalate. The intravenous injection of small amounts (0·005 gm. in a three-kilo animal) produces hypercoagulability of the blood, large amounts (0·25 gm.) completely inhibit clotting. The intravascular injection of an appropriate amount of $CaCl_2$ during the condition of incoagulability causes extensive

intravascular clotting. Similar results are obtained in those rabbits which survive oxalation. In both cats and rabbits the intravenous injection of small amounts of sodium citrate produces hypercoagulable blood, larger amounts suppress the coagulability of the blood so that it remains fluid for twenty-four hours or longer after it is shed. Recalcification *in vivo* does not provoke intravascular clotting in cats, but may or may not produce this result in rabbits. In both species the blood is rendered hypercoagulable (Pickering and Hewitt, 1925).

In cats, a partial recovery from the toxic effects of citrate occurs naturally and is accompanied by a restoration of the coagulability of the blood. A less marked increase of coagulability may occur after the hypocoagulability produced by oxalation. The natural restoration of coagulability does not provoke intravascular clotting (Pickering and Hewitt, 1925). It has been shown that citrates are eliminated from the blood-stream by oxidation (Salant and Wise, 1916–1917), and it is probable that oxalates are partially destroyed by similar action. The natural restoration of the coagulability of the blood may be thus explained. The rapid destruction of citrates *in vivo* provides a defence against the toxic action of small amounts of these salts, such as those used in blood transfusion.

Several current explanations of the stability and coagulability of the blood, particularly those associated with the names of Morawitz, Howell, Bordet and Mills, are largely based on observations on plasmas which have been either oxalated or citrated *in vitro* and then recalcified. It is implied that the behaviour of plasma after such treatment provides reliable information for the elucidation of the properties of normal blood. The observations just described show that such a conclusion may not be always true. Circulating blood in a normal condition, and even blood which has regained its coagulability *in vivo* after citration, behaves like a liquid in equilibrium. Its fluidity is preserved until it is shed. Blood which has been citrated or oxalated *in vitro* and then recalcified behaves like material not in equilibrium. It rapidly changes from a sol to a gel

and so reacts like blood which has been altered by contact-action. It appears that observations on plasmas which have been citrated or oxalated *in vitro* and then re-calcified are only of general value in the interpretation of changes in shed blood.

Salts of zinc suppress the coagulability of both circulating and shed blood, and it has been suggested that they might be used with advantage in blood transfusion instead of sodium citrate. Zinc sulphate, for example, is twenty times more active as an anticoagulant than is citrate of sodium (Lumière and Couturier, 1925, 1926). The advisability of this procedure is questioned by Mélon (1926), on account of the toxicity of zinc salts. He states, however, that non-toxic doses of zinc sulphate inhibit blood clotting in rabbits and guinea-pigs, but that this action disappears in about an hour. He believes that the activities which normally lead to the production of thrombin are restrained.

The anticoagulant actions of heparin, hirudin and of antithrombins have already been discussed. Of these bodies, the first two are useful in experimental physiology. The present state of knowledge suggests, however, that sodium citrate is the best anticoagulant for transfusions, but heparin may possibly prove of value in medical practice.

CHAPTER IX

The Condition of the Blood in Anaphylactic and Anaphylactoid States

The general characteristics of anaphylactic shock—Some explanations of anaphylaxis—Sensitisation, antisensitisation and desensitisation—Anaphylactoid phenomena—Some practical applications and suggestions for research—Addendum.

THE introduction of foreign protein into the blood-stream may evoke, after several days, an increased response to the subsequent administration of the same kind of protein. The unknown process which causes the hypersensitiveness is called "sensitisation." The effects produced by the administration of protein after sensitisation are known as "anaphylactic shock." In their most typical form there occurs marked bronchial constriction, followed by dilatation of the lungs, hypersensitiveness of the smooth muscles of certain organs and alterations in the coagulability of the blood. A phase of hypercoagulability is followed by hypocoagulability, which is manifested either by delay in the clotting of shed blood or by the complete loss of the capacity to clot spontaneously. The phase of hypercoagulability may, however, be of such short duration that it is not readily recognised. Its occurrence is of considerable importance, since it indicates that changes in the stability of the blood precede the suppression of blood clotting. Agglutination of platelets and their disappearance from the blood-stream also occur and, more rarely, thrombi are formed in the pulmonary, hepatic or cerebral vessels. This syndrome may vary both quantitatively and qualitatively. The reactions in the respiratory system and in smooth muscle may be distinct when the coagulability of the blood is hardly altered. Lee and Vincent (1917) describe this condition in cases of mild shock.

Changes in the stability of the blood do not apparently account for all the phenomena. The observations of Dale (1912–1913) on the isolated uterus of the sensitised guinea-pig suggest that the provocative dose of protein produces colloidal disturbance in the uterine muscle fibres and so evokes contraction without the intervention of blood plasma. It seems probable that Zinsser (1914) and Kopaczewski (1923) are justified in describing two reactions, one of which is "humoral," the other "cellular." But the importance of the condition of the blood is illustrated by the following experiments: (i.) Hypersensitiveness to protein shock, manifested as passive anaphylaxis, is produced when the blood of a sensitised animal is transfused into one which has not been sensitised. (ii.) A stronger bronchial reaction is produced when the isolated lungs of a sensitised guinea-pig are perfused with a mixture of blood and the same protein as that used for sensitisation than when the protein is used alone (Manwaring and Kusama, 1917). (iii.) The perfusion of a sensitised liver with the appropriate protein dissolved in blood produces shock, but perfusion with the same kind of protein dissolved in Locke's fluid does not cause any reaction (R. Weil, 1917). (iv.) It has recently been demonstrated that the intravascular injection of heparin inhibits anaphylactic shock in pigeons (Kyes and Strauser, 1926), and it has been mentioned that heparin prevents the onset of blood clotting without apparently producing any other physiological reaction in the body.

The use of a native protein, or of a grouping of amino-acids which is nearly as complex as protein, seems essential for the production of anaphylaxis. Gelatin is, however, inactive (Starin, 1918), as well as racemised proteins (Ten Broeck, 1914). Various peptides up to octopeptide are also ineffective (Hanzlik, 1924). Intravascular injection is the most effective method of administration. Mitigated anaphylactic shock has, however, been produced by intradermal injections (Vines, 1920). Similar shock-reactions have been described as occurring after the oral administration of egg-albumin to young guinea-pigs. They seem evident from the clinical observations on young children (Hanzlik, 1924) and from the experimental production of alimentary anaphylaxis (Arloing and Langeron, 1924).

Different degrees of anaphylactic shock are exhibited by animals of different species. In dogs and guinea-pigs the reaction is severe and may be accompanied by complete loss of the capacity of the blood to clot spontaneously (Arthus, 1910; Shattuck, 1917). But the changes produced by shock in these animals are not identical, the alkalinity of the plasma being increased in dogs and decreased in guinea-pigs (de Waele, 1924). In rabbits the shock may be less severe, and in some animals there is hardly any alteration in the stability of the blood. It is noteworthy that de Waele (1924) found that in some rabbits the shock is as severe as in dogs and that in these animals there is alkalinisation of the plasma. The changes in hydrion concentration are not, however, sufficient for the prevention of blood clotting.

Some Explanations of Anaphylaxis

A general discussion of the many explanations of anaphylaxis is beyond the scope of this work. Some indications of the different trends of opinion are, however, necessary for estimating the value of current views on the condition of the blood during anaphylactic shock. The earlier theories were reviewed by Zinsser (1914), and some of the later work by Wells (1921). The suggestion that a specific toxin is formed gained credence, and interpretations were sought in accordance with "Ehrlich's Side-chain Theory." Opposed to this teaching is the conclusion of Novy and de Kruif (1917) that the phenomena of anaphylaxis are not connected with those of immunity, except that they both show the response of blood to alien substances.

Several terms have been prematurely introduced to designate the "shock-producing" poison, such as "anaphylatoxin," "serotoxin," "apotoxin," "proteotoxin," and "taraxogen," but not one of these so-called toxins has been isolated or demonstrated (R. Weil, 1917; Hanzlik, 1924). There are, however, some observations which suggest the occurrence of proteolysis (Pfeiffer, 1915; Jobling and collaborators, 1915), but the changes were observed in serum and were not demonstrated in plasma. The work

of Dale (1912–1913) on the sensitised uterus of the guinea-pig renders it improbable that the material which stimulates anaphylactic contraction in unstriped muscle is a product of proteolysis, produced by enzyme-action. It is, however, generally recognised that slight disturbance of the equilibrium of blood plasma produces toxicity, and it seems probable that disturbance of equilibrium within cell protoplasm, if it involves dissociation or regrouping of colloidal complexes, may well be the primary cause of the cellular reactions.

The similarity of anaphylactic phenomena to those of histamine intoxication and the presence of the antecedent of histamine, histidine, in every known complete protein suggests that anaphylaxis arises from the liberation of histamine There are, however, several differences between histamine-shock and that of anaphylaxis. (1) Histamine fails to desensitise animals or tissues, yet produces strong reactions in thoroughly desensitised uterine muscle (Dale, 1912–1913). (2) Histamine does not produce either temperature reactions or alterations in the coagulability of the blood, which are usual in anaphylaxis (Wells, 1921). Quinine augments the susceptibility of sensitised animals to the protein shock, but does not affect intoxication by histamine (M. I. Smith, 1920).

Dale and Laidlaw (1918–1919) suggest that the similarity of histamine and anaphylactic shock is due to identical physical changes being produced in the body cells by both reactions. Both Hare (1926) and Lewis (1927) are, however, convinced that the liberation of a substance with a histamine-like action is a fundamental factor in anaphylactic reactions. The latter conclusion apparently applies only to the "cellular reactions," and not to the changes in plasma.

Other writers suggest localised action in the nervous system. Gay and Southard (1908) maintain that the respiratory centre is primarily involved. Pearce and Eisenbray (1919) favour the nerve endings. Lumière and Enselme (1926) state that anæsthesia of the endovascular nerve endings suppresses anaphylactic shock. They conclude that changes in plasma excite nervous reactions, which produce shock. Lumière and Couturier (1921) regard cerebral activity as essential. But Zunz and La Barre (1926) found that decerebration and even removal of the

bulb does not prevent the changes in the blood which characterise anaphylactic shock. From observations on animals deprived of the blood supply to various organs, Manwaring (1911) concluded that hepatic action is essential. A similar conclusion was reached by R. Weil (1917) and has been accepted by many writers. Protein shock, without sensitisation but in other respects almost indistinguishable from anaphylactic shock, can, however, be produced with the liver out of the circulation (Nolf, 1921). In a more recent paper, Manwaring (1924) assumes that the capillary endothelium is the site of anaphylactic reactions. In dogs the hepatic endothelium is the most susceptible and permits the rapid passage of foreign protein and of altered blood plasma into the liver. New products are formed which pass into the blood-stream and attack the endothelium of other organs and produce secondary reactions. Differences in the susceptibility of different animals are explained as due to variations in the condition of the endothelium.

Recent investigations on the physical condition of plasma during anaphylaxis have led to the conclusion that the changes arise from the flocculation of body colloids. Both the plasma and body cells are believed to be involved (Kopaczewski, 1921, 1923; Hanzlik, 1924; Blum and collaborators, 1924; Lumière, 1926). Besides flocculation of the plasma, changes in its surface tension, in its ionisation and in its viscosity have been observed (Kopaczewski, 1923; Zunz, 1925; Milkovitch, 1926; Waud, 1927). It is not, however, established that these changes suffice for the production of anaphylactic shock. They may merely indicate other alterations in the plasma, such as colloidoclasis which may produce the physiological reactions.

In many respects the condition of the blood during anaphylactic shock resembles that produced by the intravascular injection of "peptone." The shed blood is clotted by the passage of CO_2, by dilution with water, by acidification (Arthus, 1910) and by the addition of tissue extracts. Its capacity to clot ultimately is destroyed by passage through a Berkefeld filter (Zunz and La Barre, 1925). The amount of fibrinogen obtainable from the plasma is com-

monly decreased, but may be substantially increased (Zunz, 1926). A stage of hypercoagulability precedes hypocoagulability. The capacity of the plasma to agglutinate platelets is increased and may be evident *in vivo*. Parallelism of behaviour is also apparent in the suppression of anaphylactic and "peptone" shock. The previous intravascular injection of hirudin, of sulphate of atropine or of chlorhydrate of choline prevents or mitigates both these reactions (Zunz, 1925). An important difference is, however, suggested by the observations of Moldavan and collaborators (1923), that blood shed from the carotid artery of the guinea-pig during the height of anaphylactic shock may be hypercoagulable, whilst that obtained from the jugular vein is hypocoagulable. In guinea-pigs, dogs and cats the intravascular injection of "peptone" produces hypocoagulability in both arterial and venous blood.

Several explanations have been given of the suppression of the coagulability of the blood during anaphylaxis. The suggestion of Nolf (1911) that it is primarily due to defibrination is inconsistent with the finding that the fibrinogen may actually be increased (Zunz, 1926). The presence of an excess of antithrombin in the blood of all animals suffering from anaphylactic shock is not established. Shattuck (1917) could not find any greater amount than can be obtained by the disintegration of normal plasma. Working with the plasmas of guinea-pigs, rabbits and cats, Bulger (1918) found that anaphylactic suppression of coagulability is associated with a decrease of antithrombin, estimated by Howell's method. Using the same method, Zunz and La Barre (1925, 1) found a marked increase in dog's plasma and concluded that this largely accounts for the loss of coagulability. It is improbable that there was any large error in the estimations of either of these observers. It thus appears probable that the anaphylactic reaction varies in animals of different species and that conclusions deduced from phenomena in the dog have only a limited meaning. In this connection the explanation offered, in the preceding chapter, of the action of "peptone" on the dog should be considered.

In concluding a review of this subject, Zunz and La Barre (1925, 1) suggest that the fibrinogen of blood plasma may be altered by the introduction of foreign protein into the blood-stream. They base this suggestion on the finding that the pre-clot changes in normal blood are reversible (Pickering and Hewitt, 1921; Kugelmass, 1923), and they cite certain observations (by Pickering and Hewitt, 1921, 1922, and Pickering and de Souza, 1923) which they suggest may imply that the fibrinogen of circulating blood is altered by various influences. A similar suggestion, unsupported by experimental evidence, was made by Novy and de Kruif (1917). Zunz and La Barre also suggest that investigation should be directed to ascertaining the condition of fibrinogen during anaphylaxis. With this remark I agree, but it seems more probable that our observations point either to differences in the stability of the union of fibrinogen with other plasma complexes or to differences in the degree of dissociation of fibrinogen from plasma.

It has been mentioned that there are marked differences in the stability of the diluted plasmas of animals of different species, those of the guinea-pig, the dog and rabbit being less resistant to salt-precipitation than are the plasmas of man, of the horse and the sheep (see Table II., p. 30). The least resistant are the plasmas of the dog and guinea-pig, and it is these animals which are very susceptible to anaphylactic shock. It has also been mentioned that much of the prothrombin of normal plasma is tightly bound to fibrinogen and so resists the changes which inaugurate clotting; also that a small amount of prothrombin is loosely bound and readily generates thrombin. The occurrence of hypercoagulability and of flocculation during the inception of anaphylactic shock implies the disturbance and dissociation of plasma colloids; the subsequent hypocoagulability shows that an exceptionally stable state of equilibrium has been reached. It is possible that the stable condition is produced by the loosely bound prothrombin being temporarily separated from fibrinogen and then uniting firmly with fibrinogen. This suggestion is consistent with the presence of an abundance of prothrombin in anaphylactic

plasma and with the almost complete absence of thrombin. Another reaction involving the stabilisation of the fibrinogen-prothrombin complex may occur if anticoagulants are liberated by autolytic processes. To test the value of these suggestions, attention should be directed to the stability of the fibrinogen-prothrombin complex during the hypercoagulable and hypocoagulable phases of anaphylactic shock.

Sensitisation, Antisensitisation and Desensitisation

Very little is known of the changes which produce sensitisation. According to Waud (1927) the blood exhibits no departure from normality until the time is reached when a second injection provokes an anaphylactic reaction. At this juncture the viscosity of the blood is slightly increased. Attempts should be made to ascertain if there are any indications of alteration in the stability of plasma colloids during the time between the administration of a sensitising dose and the production of hypersensitivity to protein shock. An investigation of the resistance of the plasma to salt precipitation after dilution might answer this question.

Antisensitisation, or the production of resistance to sensitisation, has been described by R. Weil (1913), and may be exhibited in several ways. If a guinea-pig is given a single dose of a serum obtained from a rabbit immunised to a particular protein several days before a sensitising dose of that serum is administered, the usual passive sensitisation does not occur. Refractoriness to sensitisation may also occur when a large sensitising dose is given (Wells, 1921). Another form of interference with sensitisation is produced when the sensitising protein is injected together with, or even twenty-four hours after, a much larger dose of another protein (Lewis, 1915). Antisensitisation has also been produced by feeding guinea-pigs on a selected protein diet (Wells, 1911, 1921). It then appears that there is a natural defence against alimentary anaphylaxis. If protein normally passes unchanged from the alimentary canal into the blood-stream, antisensitisation may account for the rarity of auto-sensitisation.

Animals which have just recovered from anaphylactic shock resist intoxication by a further injection of the same kind of protein. The physiological process producing this result is called "desensitisation." The resistance may be either complete or partial. After shock has been produced by a minimal amount of protein, the animals still react to another injection. Desensitisation by anaphylaxis is a strictly specific reaction. For example, if a guinea-pig is sensitised to two different kinds of protein and recovers from the shock produced by one of them, to which it becomes completely resistant, it will still react to the second protein, but the reaction is much less severe than when desensitisation to the other protein has not been produced. These phenomena are so sharply defined that they have been used in differentiating blood-stains (Thomsen, 1908–1909), and in determining the purity of proteins (Wells, 1921). Desensitisation can be produced without any severe reaction if the desensitising dose is introduced slowly enough, as when subcutaneous injection is used.

These occurrences have been explained from the immunological standpoint by postulating exhaustion of antigen (Wells, 1921). It seems equally probable that the body colloids remain in an exceptionally stable condition for some time after the cessation of the more patent manifestations of anaphylaxis and so resist disturbance by the alien protein. This suggestion is supported by the fact that increased stability of the blood, manifested by hypocoagulability without depletion of plasma colloids, may continue for a considerable time (one or two hours in rabbits) after the animals have apparently recovered from anaphylactic shock.

A wide range of substances prevents or mitigates anaphylactic shock when injected into the circulation of sensitised animals just before the provocative dose is given and thus behave as desensitising agents. Included in the list of these bodies are carbonates and bicarbonates (Kopaczewski and Roffo, 1920; Sicard and Paraf, 1921), oleate of soda (Kopaczewski and Vahram, 1919), foreign protein other than that used for sensitisation, peptones, trypsin (Wells,

1921), hirudin (Zunz and Van Geertruyden-Bernard, 1925), atropine sulphate and chlorhydrate of choline (Zunz, 1925). Adrenaline mitigates the bronchial spasm, but may not prevent the occurrence of hypocoagulability of the blood. Heparin is a desensitising agent when injected into pigeons (Kyes and Strauser, 1926), but increases the severity of anaphylactic shock in guinea-pigs (Waud, 1927).

Desensitisation by carbonates or bicarbonates has been attributed to the increase in the viscosity of the blood which follows the introduction of these salts into the circulation (Kopaczewski and Roffo, 1920). That produced by sodium oleate has been ascribed to the lowering of the surface tension of the plasma (Kopaczewski, 1923). It may be added that the ability of hirudin to mitigate both anaphylactic and "peptone" shock is proportional to its capacity to lower the surface tension of the blood (Zunz, 1925, 2). But it has not been shown that all desensitising agents act similarly.

Broadly speaking, the phenomena of antisensitisation and of desensitisation support the view that the changes produced by the provocative dose in anaphylactic shock may occur in the blood and that desensitisation may also arise from a humoral reaction. The recent experiments of Girard and Peyre (1926, 1, 2) point in the same direction. These investigators found that the intravenous injection into rabbits of an approprate amount of eosinate of cæsium prior to the injection of a sensitising protein mitigates or prevents the subsequent production of anaphylaxis. Moreover, this substance produces a granular deposit in plasma, and so isolates certain colloids which participate in the inception of anaphylactic phenomena.

The different results obtained when heparin is injected into sensitised pigeons and guinea-pigs, and a provocative dose of protein then given require, however, explanation. In both guinea-pigs and pigeons heparin first produces hypocoagulability of the circulating blood, and later hypercoagulability. In the former animals the decreased coagulability lasts for fifteen to twenty minutes, in the latter for thirty to a hundred minutes. It is possible that the more rapid onset of hypercoagulability in the guinea-pig

may account for the failure of heparin to suppress anaphylaxis in these animals.

Anaphylactoid Phenomena

Effects closely resembling anaphylactic shock are rapidly produced when certain substances are introduced into the blood-stream, even when sensitisation has not been produced. Foreign protein, when injected intravenously in larger amounts than are required for the production of shock after sensitisation, produces typical results, and less severe reactions may follow intra-peritoneal injections. Similar effects are produced by the intravascular injection of appropriate amounts of the following bodies :—various non-nitrogenous colloids (such as agar, tragacanth, pecten, gelose and starch), arsphenamine, tissue extracts, proteoses, peptones, salts of heavy metals, colloidal metallic preparations (iron and collargol), some coal-tar derivatives, trypanosomes, tuberculin, vaccines, adsorbents and hypertonic solutions (Hanzlik, 1924). Antipyrine and quinine will produce shock in guinea-pigs, even when administered orally (Arloing and collaborators, 1924). Injurious reactions associated with definite changes in the physical or chemical condition of the blood may also follow the intravenous injection of urotropine, sodium iodide and sodium salicylate (Hanzlik, 1924).

Different degrees of susceptibility to anaphylactoid shock are exhibited by animals of different species, and the effects vary with the dosage and mode of administration. According to Hanzlik (1924), the coagulability of the blood is either unchanged or inhibited, the result depending on the nature of the substance employed and on the extent of the agglutination and destruction of platelets or on both these occurrences.

The changes that produce these reactions are still obscure. Both flocculation and a fall in the surface tension of the plasma have been observed (Hanzlik, 1924 ; Zunz, 1925). Disturbances in the physical or chemical condition of the plasma are thus apparent, and these may well produce disintegration of cells and solid tissues. It is noteworthy

that changes in the viscosity and surface tension of the plasma occur after the intravenous injection of sodium salicylate, when there is hardly any alteration in the coagulability of the blood.

The differences in the conditions governing the suppression of blood clotting by the injection of " peptone " and tissue extracts suggest, however, that more than one sequence of events may produce anaphylactoid shock. In the case of the shock produced by the intravascular injection of heterologous blood, there is some indication that changes in the blood are involved which are similar to those which initiate clotting. Novy and de Kruif (1917) found that if a quantity of the blood of a rabbit, up to 3 c.cm. in amount, is injected immediately into the circulation of a guinea-pig, it is usually harmless, but if the same amount of blood is left in a glass syringe for three minutes or longer, and then injected, the results produced resemble those occurring in anaphylactic shock. It may be added that contact of rabbit's blood for three minutes with glass produces disintegration of platelets and converts some of the prothrombin of the plasma into thrombin, but does not produce clots.

Some Practical Applications and Suggestions for Research

The experimental production of sensitisation and of anaphylactic shock has led to the avoidance of these reactions in serum therapy. But accidents still occur when direct sensitisation is not apparent and when the dosage does not exceed that commonly used. The individuals are hypersensitive to protein shock. In such cases an investigation of the physical condition of the plasma by the methods of Kopaczewski (1921, 1923) and the testing of its stability by its capacity of resisting salt-precipitation after dilution might provide the information for the avoidance of the harmful reactions. Similar modes of research should also be applied to the investigation of anaphylactoid asthma, of urticaria, of " alimentary anaphylaxis," and of anaphylactoid purpuras.

The most useful lesson taught by anaphylactoid pheno-

mena is provided by the demonstration of the danger of intravenous injection. It should be noted that several substances which may provoke anaphylactoid effects when injected intravenously are still administered by this method in medical practice, even when beneficial results, without shock, follow oral administration. It should also be noted that it is not yet possible to predict the physiological reactions following intravascular injection from the chemical or physical properties of the substance injected or from the results following oral administration. Much valuable work has been done by Hanzlik and his collaborators (1919–1924) in scheduling medicaments which are unsuitable for use intravascularly, but no new substance should be injected into the circulation until it has been fully tested on animals of different species for anaphylactoid reactions. In this connection two new facts may be mentioned. In anæsthetised cats and guinea-pigs in which the blood has been rendered hypercoagulable (either by the introduction of strings into the circulation, or by the intravascular injection of thrombin), the intravenous injection of an amount of rabbit's serum insufficient to produce anaphylactoid shock in normal animals may produce dyspnœa, agglutination of blood platelets and hypocoagulability of the blood. Similar, but less severe, reactions may follow intradermal injections. It appears that hypercoagulability of the blood favours the production of anaphylactoid shock, and when this condition occurs in man, as in thrombophlebitis and after severe operations, serum therapy appears contra-indicated.

Much discussion has arisen respecting the frequency of true anaphylactic reactions in man, and their relationship to the hypersensitiveness manifested by cutaneous reactions, to some forms of asthma, and to other allergic phenomena. In certain acute serum reactions, as in one in which a person suffering from " horse asthma " dies in a few moments after the injection of horse serum, there is no doubt that typical anaphylaxis occurs. Passive sensitisation has also been observed when the blood of an affected person has been transfused into a normal individual (Ramirez, 1919). In " horse asthma " and kindred conditions the intradermal

injection of very minute amounts of the appropriate protein produces a cutaneous reaction. There is then a *primâ facie* case showing a relationship in man between cutaneous reactions and anaphylaxis, and the relationship of certain cases of serum sickness to anaphylaxis appears established, particularly by the observations of C. W. Wells (1915). Bovine sera seldom cause serum sickness (Penna, 1918), and this may be due either to antisensitisation or to desensitisation arising from the use of beef as food.

Desensitisation by appropriate feeding in the treatment of food allergy affords another example of the practical application of facts disclosed by the investigation of anaphylaxis. The investigation of the effects of a high protein diet on those allergic phenomena which are amenable to desensitisation might also provide useful information.

The resemblance of serum sickness to anaphylaxis is, however, often indistinct. Both the general and the cutaneous reactions may occur after varying intervals of time, and may immediately follow the first injection. In the latter case there is a greater resemblance to anaphylactoid phenomena than to anaphylactic shock. That these two forms of disturbance may react differently to desensitising agents appears evident from the observations of Karsner and Hanzlik (1920). The bronchial spasm in anaphylactoid shock is not controlled by adrenaline or atropine, and so differs from that of anaphylactic shock. An investigation of the action of non-specific desensitising agents in serum sickness might prove of value.

The condition of blood plasma in many forms of asthma which have been classed as "allergic phenomena" remains almost completely obscure. The observations that the symptoms of asthma can be produced by the inhalation of *p*-phenylene-diamin (Gerdon, 1920), together with the diversity of bodies which produce cutaneous reactions, including non-protein substances (Van Leeuwen, 1925), illustrate the dubiety of the position. The application of the methods of research mentioned earlier in this chapter might, however, provide a means of differentiating some allergic conditions which exhibit similar symptoms.

Several pathologists have suggested that a relationship exists between eclampsia and anaphylaxis. Of these writers, Esch (1912) and Franz (1912) claim the presence of "anaphylactic poisons" in the serum and urine of eclamptics. The existence of such a relationship is, however, opposed by a number of other investigators, particularly by Zweifel (1921), whose work suggests that eclampsia is not an anaphylactic reaction in which either the fœtal or placental proteins participate. A novel hypothesis has recently been promulgated by Hynd (1925). From an investigation of the urinary protein in cases diagnosed as eclampsia, he suggests that the urinary albumin in that condition is lactalbumin and that certain types of eclampsia are due to an anaphylactic reaction arising from the entry of lactalbumin into the blood-stream. The absence of eclampsia in most cases where a mother does not suckle her child is explained as due to "immunisation," but either "antisensitisation" or "desensitisation" might be postulated. Further investigations are, however, required to test the value of these suggestions.

Recent observations suggest that renewed investigation of antisensitisation may throw a new light on the response of the body to toxic substances. Animals which have just recovered from anaphylactic shock resist intoxication by strychnine, the convulsions produced by that drug being either wholly or partly arrested (Arloing and Langeron, 1924; Claude and Montassut, 1927). It is also stated that the injection of colchicin into guinea-pigs three days before sensitisation prevents or mitigates anaphylactic shock, and that a similar reaction does not occur when the colchicin is injected just before sensitisation (Arloing and Langeron, 1926). The parallelism of behaviour to the action of colchicum in gout can hardly be without significance.

Addendum.—An important paper on anaphylactoid phenomena was published by Hanzlik and his collaborators (1928) after this chapter was written. Increases are recorded in the *albumin ratio* of plasma which are reciprocal to the globulin alterations in surface tension. These writers maintain that the fundamental cause of anaphylactoid shock is the disturbance of the physical and chemical equilibria between body fluids and cells. They point out that their observations are consistent with conclusion that hæmoclasia or colloidoclasia participates in the disturbances of allergic phenomena in general.

CHAPTER X

Current Theories of Blood Coagulation

The conclusions of Wooldridge, Morawitz, Nolf, Bordet, Howell, Hekma Mills, and of the present writer.

MODERN explanations of blood clotting fall into two groups. Most investigators maintain that the action of thrombin on fibrinogen is the starting point of gel-formation in the plasma. But the followers of Wooldridge, particularly Nolf and Mills, believe that although thrombin is produced when blood clots, it is of secondary importance in that process. It is, however, generally admitted that calcium ions are essential for the inception of clotting and that active thrombin does not exist in normal circulating blood. The participation of bodies liberated by the lysis of blood platelets and of tissue cells is also generally accepted. But on almost all other questions there are fundamental differences of opinion.

The hypotheses of Wooldridge and of Morawitz are briefly mentioned, although they are now discarded by almost all research workers. The former theory provides, however, the basis upon which Nolf has built his opinions, the latter is still used by pathologists in explaining abnormal conditions of blood plasma.

The Theory of Wooldridge

Blood plasma is said to contain all that is necessary for its coagulation. Clotting is explained as due to the interaction of two plasma constituents, named A and B fibrinogen, which unite to form fibrin. The fibrinogen known in the laboratory is stated to be almost absent from the plasma, but to be formed from B fibrinogen when blood is shed. Thrombin is regarded as a subsidiary reaction-product. Blood platelets are described as artefacts, produced by the

cooling of plasma. But tissue juices are believed to take the place of A fibrinogen in clotting, and the intravascular coagulation following their injection is so explained. Wooldridge attached much importance to the action of crude lecithin in clotting and thus foreshadowed the recognition of the *rôle* of phosphatides in coagulation.

Subsequent research has partly justified these conclusions, but it has shown that some of them are erroneous. It has established that platelets are actual formed elements of the blood, and that clotting without their intervention, except during the height of digestion and in certain abnormal states, is so slow that the plasma appears incoagulable at room temperatures. There is also little doubt that A fibrinogen contains the *débris* of platelets, and that B fibrinogen is a fraction of plasma containing fibrinogen and prothrombin.

The Theory of Morawitz

According to Morawitz (1904), both fibrinogen and the mother-substance of thrombin (thrombogen) exist in plasma. When blood is shed the lysis of leucocytes and of platelets liberates an enzyme (thrombokinase), which with the help of calcium ions converts thrombogen into thrombin. The latter body, also by enzyme action, alters fibrinogen into fibrin. Although the evidence already collated discredits the conclusion that enzymes are essential factors in normal blood clotting, the possiblity of catalysis in the inception of coagulation should not be ignored. Such activity seems apparent in the intravascular clotting produced by certain snake venoms and contact-catalysis may participate in the lysis of plasma complexes and of platelets in shed blood.

The Theory of Nolf

I am indebted to Professor Nolf for the following summary of his conclusions :—

P. Nolf admet, en accord avec une opinion formulée par Wooldridge à propos du plasma des mammifères, qu'il élargit, que le plasma de tous les vertébrés contient en abondance toutes les substances mères de la thrombine et de la fibrine.

Cette opinion est basée sur un certain nombre de constatations faites sur différents plasmas stables, recueillis purs de toute souillure par du suc de tissu et débarrassés par une ou plusieurs centrifugations de tout élément figuré.

1° Le plasma normal des poissons marins reste indéfiniment fluide au contact du verre. Il se coagule rapidement par l'adjonction d'une suspension de verre porphyrisé ou par simple dilution dans l'eau de mer.

2° Le plasma de peptone (plasma d'un chien ayant reçu 0·1 gr. de peptone par kilogramme dans une veine) reste indéfiniment fluide au contact du verre. Il se coagule en quelques minutes, si on lui ajoute une petite quantité d'oxalate sodique et son équivalent de chlorure calcique de façon à provoquer la formation d'un précipité colloidal d'oxalate calcique.

3° Le plasma normal d'oiseau reste indéfiniment fluide au contact du verre. On le coagule en quelques minutes, en lui mélangeant à l'état d'émulsion une petite quantité de chloroforme (la cinquantième partie de son volume).

Ces faits n'ont jamais été mis en doute par personne.

Vouloir expliquer cettes coagulations en supposant que malgré toutes les précautions prises, les plasmas précités étaient souillés par des traces de suc de tissu ou qu'ils contenaient un petit nombre d'éléments figurés du sang, malgré les centrifugations énergiques qu'ils avaient subies, c'est :

1° faire une hypothèse gratuite qu'aucun fait précis n'a appuyé. 2° oublier que ces liquides abandonnés à eux-mêmes restent indéfiniment fluides au contact du verre. Or cette fluidité a été attribuée par A. Schmidt et ses continuateurs à l'absence d'un élément appelé par A. Schmidt substance zymoplastique et que ses continuateurs ont reconnu être un lipoïde apparenté à la lécithine. Pour eux, ce lipoïde est un élément indispensable à la coagulation. Il est absent du plasma normal ; par contre il appartient aux éléments figurés du sang (leucocytes et plaquettes) et en général aux cellules des tissus. Pour interpréter les expériences relatives aux trois plasmas stables précités, il faut en même temps supposer que le lipoïde est absent de ces liquides, puisquils sont spontanément incoagulables, et présent, puisque sans lui il n'y a pas de coagulation possible par la poudre de verre l'oxalate calcique ou le chloroforme.

A vrai dire, on pourrait supposer qu'il est présent en quantité suffisante pour permettre la coagulation sous l'influence d'un facteur adjuvant et insuffisante pour que la coagulation du plasma soit spontanée. Mais les expériences suivantes vont directement à l'encontre de cette manière de voir : Si l'on détermine la richesse en thrombine d'un sérum, en recherchant quelle est la concentration limite du sérum qui est encore capable de coaguler un volume déterminé de solution oxalatée de fibrinogène, on constate que le

sérum complet du sang d'oiseau est beaucoup moins riche en thrombine que le sérum obtenu par la coagulation du plasma pur d'oiseau sous l'influence du chloroforme. Ce résultat ne s'accorde pas avec l'opinion que la thrombine du sang complet s'est formée par l'union d'un élément fourni par les leucocytes et d'un élément fourni par le plasma.

On peut d'autre part provoquer la coagulation de deux échantillons du même plasma pur d'oiseau en ajoutant au premier la dose optime du lipoïde coagulant et au second du chloroforme en émulsion. On constate que le sérum du second contient jusque cent fois plus de thrombine que le sérum du premier. P. Nolf a conclu de ces faits que le lipoïde coagulant n'apporte au plasma aucune substance-mère de la thrombine mais que son rôle est analogue à celui du chloroforme : l'un et l'autre facilitent la coagulation du plasma, ils exercent tous deux une influence thromboplastique.

Ces expériences apportent la preuve non réfutée que la plasma pur contient toutes les substances-mères de le thrombine.

Ce point est essentiel. Aussi longtemps que l'accord ne sera pas fait en physiologie sur la question de savoir si le plasma contient ou non toutes les substances-mères de la thrombine et de la fibrine, on continuera à discuter à perte de vue sur la nature du phénomène. Il est clair que suivant qu'est vraie la théorie uniciste (présence dans le plasma de toute les substances-mères de la fibrine et de la thrombine) ou la théorie dualiste (absence du plasma d'une des substances-mères) tous les problèmes de la coagulation : fluidité du sang dans les vaisseaux, mécanisme de la coagulation, etc., se posent différemment.

Wooldridge admettait que les colloïdes protéiques du plasma qui prennent part à la coagulation du sang sont deux : le A-fibrinogène et le B-fibrinogène. Dans son opinion, la coagulation est une réaction entre ces deux substances qui les consomme l'une et l'autre. Comme Wooldridge, P. Nolf soutient que la formation de la fibrine est due à l'union de plusieurs colloïdes. Mais il se sépare de Wooldridge en affirmant que ces colloïdes sont trois. Le premier, qu'il a obtenu à l'état de pureté, est le thrombozyme. Elle est l'analogue de l'A-fibrinogène de Wooldridge. Wooldridge avait reconnu que l'A-fibrinogène coagule le plasma de peptone mais ne coagule pas la solution de fibrinogène de Hammarsten. Il en avait conclu que le fibrinogène de Hammarsten est le B-fibrinogène dénaturé du plasma. L'ensemble des faits actuellement connus tend à faire croire que le fibrinogène de Hammarsten est un élément normal du plasma. Mais ainsi que P. Nolf l'a montré, il est incapable de s'unir à la thrombozyme aussi longtemps qu'il est seul en présence de cette substance. Pour que l'union soit possible, il faut une troisième substance protéïque qui existe abondamment dans le plasma, mais n'a

pas pu en être isolée, le thrombogène. La formation de la fibrine est due à l'union en un seul complexe de la thrombozyme, du thrombogène et du fibrinogène.

Le B-fibrinogène de Wooldridge correspond donc à un mélange de thrombogène et de fibrinogène de Hammarsten.

Des trois substances-mères de la fibrine, le fibrinogène et le thrombogène sont produits par le foie. La thrombozyme provient des endothelia vasculaires extra-hépatiques, de leucocytes, des ganglions lymphatiques.

La coagulation étant l'union de la thrombozyme avec deux protéines hépatiques est grandement influencée, comme toutes les unions entre colloïdes par toute une série de facteurs, comme le *p*H, l'ionisation, les proportions des colloïdes actifs en présence, l'abondance plus ou moins grande de colloïdes protecteurs (antithrombosine), etc. Un grand nombre de substances solubles ou insolubles, comme la poudre de verre, l'oxalate calcique colloïdal, le lipoïde coagulant, le chloroforme, etc., favorisent cette union. Ce sont les substances thromboplastiques.

Quand on traite un plasma de mammifère par un précipité gélatineux de phosphate tricalcique ou d'hydrate d'alumine, on lui enlève la plus grande partie de sa thrombozyme, tout en lui laissant son fibrinogène et ses protéines hépatiques. Après ce traitement, le plasma a perdu la propriété de se coaguler spontanément. Il peut même être faiblement anticoagulant. Ceci démontre que la stabilité d'un plasma est due à la prédominance de protéines hépatiques sur la thrombozyme.

D'un plasma à l'autre, les différences de stabilité dépendent du rapport variable entre la thrombozyme et les protéines hépatiques.

La thrombine prend naissance par l'union de la thrombozyme et du thrombogène. Elle est une fibrine incomplète qui se sature en se combinant au fibrinogène.

La thrombozyme est une enzyme protéolytique qui hydrolyse les protéines hépatiques, après les avoir coagulées.

The Hypotheses of Bordet

Bordet (1920) explains the maintenance of the fluidity of circulating blood as due to the inactivity of the material which participates in the formation of thrombin. He believes it is stabilised either by a specific antibody or by association with protective colloids. Whenever blood is shed upon a surface which it wets, contact-action converts the inactive substance (proserozyme) into active material (serozyme). The latter body unites with the phosphatides of blood

platelets and of tissue juices and produces thrombin, which in turn unites with fibrinogen and forms fibrin. There is no enzyme-action and the combinations occur in varying proportions, in a similar manner to the unions of toxins and antitoxins.

These conclusions are largely based on the stability of prothrombin in plasma which has been exposed to adsorption by finely divided tricalcium phosphate, and it has recently been suggested that the acidity of Bordet's plasma accounts for its stability (Maltaner and collaborators, 1927) It can, however, be demonstrated that plasmas which have been deprived of a small part of their protein by exposure to animal charcoal retain their stability in neutral solution.

The Theory of Howell

Professor Howell has summarised his conclusions as follows :—

" The central point of Howell's views on coagulation is that the normal fluidity of the blood is maintained by the presence of an inhibitory substance In his early papers (1911), he identified this substance with the antithrombin which is known to exist in blood in small concentrations It is a substance of unknown chemical nature analogous in its action to hirudin in that it prevents the reaction between fibrinogen and thrombin. Subsequent work did not tend to support this view of the function of the blood antithrombin, particularly in regard to the antagonistic influence of tissue extracts. In his later papers, Howell (1925–1926) gives evidence for the presence in the blood of an inhibitory substance of an entirely different nature. This substance, which can be obtained most readily from the dog's liver, is a carbohydrate body, apparently a compound of glycuronic acid, to which the name heparin has been given. It is an extremely potent anticoagulant In its purified form one milligram will prevent the clotting of as much as 100 c.c. of human blood. It differs in its action from hirudin or blood-antithrombin in that it has no effect upon the reaction between fibrinogen and thrombin. Its effect in preventing coagulation of blood is due to the fact that in some way it prevents or retards the formation of thrombin, that is to say, it may be considered to act as an antiprothrombin rather than as an antithrombin. In addition to this action it has the property, when added to blood or serum, of causing the formation of an increased amount of blood-antithrombin. Howell has shown that a substance similar in its properties to heparin exists in normal blood, and in much larger quantities in the

incoagulable blood of peptonised dogs. He believes, therefore, that this substance is probably formed normally in the liver and is given off to the blood in concentrations sufficient to prevent the conversion of prothrombin to thrombin. When blood is shed the thromboplastic material given off by the disintegrating platelets in mammalian blood, or by the extravascular tissues, serves to neutralise the effect of the heparin and thus permits the activation of prothrombin to thrombin. In regard to the nature of the thromboplastic substance present in tissue extracts and particularly in blood-platelets, Howell has shown that the essential constituent is one of the phosphatides, cephalin, which has the specific property of initiating the process of coagulation as it normally occurs. In all probability cephalin in the tissues is combined with protein as a protein-cephalin compound, but the isolated cephalin itself has to a marked degree the property of hastening or initiating the process of clotting. Howell holds, therefore, to the traditional view that the chemical process of coagulation takes place in two stages: first, the conversion of prothrombin to active thrombin; second, the formation of fibrin by a reaction between thrombin and fibrinogen. In addition he emphasises a third physico-chemical stage, namely, the gelatinisation of the liquid that takes place after the fibrin is formed. The activation of prothrombin to thrombin may take place spontaneously under certain conditions, or it may be effected experimentally by a number of different reagents (Cekada, 1926), chloroform, alcohol, etc., but in the blood normally this activation is induced by calcium salts. In the circulating blood both prothrombin and calcium are present. Howell believes that the activating effect of the calcium is prevented normally by the presence of heparin, but that when thromboplastic substance (cephalin) is added in sufficient amounts the inhibitory action of the heparin is neutralised, and the calcium then is able to convert the prothrombin to thrombin. In regard to the last stage of blood-coagulation, the process of gelatinisation, he and others have shown that fibrin when formed normally is deposited as needle-shaped crystals (Howell, 1914, 1916). The solidification of the blood to a jelly is apparently a subsequent reaction, the nature of which is not known. In a condensed form the theory assumes: (1) That the fluidity of the circulating blood is maintained by the presence of an inhibitory substance, heparin, which prevents the conversion of prothrombin to thrombin. (2) When blood is shed the blood-platelets disintegrate with the liberation of a cephalin compound (thromboplastic substance), which neutralises the heparin and thus permits the calcium to activate the prothrombin to thrombin. A similar thromboplastic substance may be furnished by other tissue cells. (3) The thrombin reacts with fibrinogen to form an insoluble fibrin,

which is deposited as needles. (4) By a reaction of the fibrin with the external phase gelatinisation takes place."

The reasons for suspending judgment in the acceptance of of some these conclusions have been given in Chapter V.

The Theory of Hekma

Dr. Hekma expresses his conclusions as follows :—

" Fibrinogen is present in blood plasma. Under the influence of hydroxyl ions, it exists in a sol-like condition in the form of hydrated micellæ. After dehydration, the micellæ of fibrinogen become unstable and micellar crystallisation occurs. The dehydrated micellæ form thread-like needles by automatically uniting lengthwise. The needles then join up lengthwise and form threads and filaments in which the original needles cannot be distinguished. A gel is so formed. It is pliant, extensible, slightly elastic and hardly viscous. It is soluble in dilute acids, in alkalis and in some salts.

Fibrin is fibrinogen and thrombin united by adsorption. Thrombin is a viscous substance with specific qualities—an agglutinin.

When leucocytes and platelets are destroyed, cytocym (cytozyme) enters the plasma. It is also probably liberated from the cells of the vascular endothelium and from some bacteria. Cytocym is an agglutinin. It forms complexes with the albumin of plasma which pass into serum, and may there exhibit agglutinative power, but to a smaller extent than the original material. Cytocym is taken up by acid bodies and may then combine with calcium, its viscosity and agglutinative power being increased.

The fibrinogen-agglutinin adsorption compound exhibits the same tendency as fibrinogen to the production of lengthy formations, but this is partly counteracted by the viscous property of the agglutinin. The micellæ of fibrinogen and the micellar crystals can absorb agglutinin when in a hydrated fluid condition and when in a dehydrated half-solid condition. In the former case, when the conditions are favourable, a gel is formed which is composed of hydrated micellæ. In the latter case, coagulation is due to the micellæ and micellar crystals uniting lengthwise. When the former mode of change predominates, fibrin appears as needles or threads. If agglutination is more active the fibrin forms irregular masses, analogous to those found in bacillary agglutination. Between these extremes, all kinds of variations occur. The agglutinin behaves like a cement and its action is enhanced by calcium.

Gels of fibrin are distinguished from gels of fibrinogen by the

adsorption of agglutinin and are so rendered extensible, elastic, contractile and viscous. The extraction of fibrin gels with water removes the agglutinin and the gel loses its viscosity. Fibrin gels are reversible when treated with acids or alkalis. Solution takes place very slowly, especially when the gel contains calcium. By the deprivation of calcium, fibrin gels can be split into fibrinogen and agglutinin. For this reason, the solution of fibrin gels containing calcium is effected much more readily by acids than alkalis. Hence, during solution the agglutinin must be more or less completely separated from fibrinogen. By employing an appropriate method, such as that of Hammarsten, the separation may be ultimately so complete that an almost pure and practically stable solution of fibrinogen remains."

The work of Hekma confirms and extends the observations of Stübel and of Howell on the morphological development of fibrin aggregates. It is consistent with the conclusions that cytozyme unites with a plasma constituent and forms thrombin, and that this product when united with fibrinogen gives fibrin. But it is difficult to believe that the initial act in blood clotting is dehydration, and the observations of Howell (1916) show that thrombin and fibrinogen can combine under conditions which preclude the union of oppositely charged particles.

The Theory of Mills

For several years the present writer has advocated that the initial change in blood coagulation is the lysis of the colloidal complexes of plasma. It will be seen that Mills has reached a similar conclusion.

" Blood plasma, although containing all the elements necessary for thrombin clotting (prothrombin, cephalin and calcium), retains its fluidity *in vivo* because of the state in which these clotting factors are held. There undoubtedly exists a union, probably molecular, between the fibrinogen, prothrombin, cephalin and antithrombin in the living plasma, each of them being held un-ionised, so that their characteristic functions in clotting are not present. Thus one cannot activate the prothrombin of unchanged plasma, nor can one demonstrate an antithrombic activity, but as soon as clotting has been initiated and the molecular complex of the plasma disrupted, then both factors display their true characteristics.

To clot plasma alone, then, one must introduce some dis-

turbing, or ionising, force which will disrupt the molecular complexes. Glass, dust, feathers, metals, etc., are such forces commonly used in laboratory experiments, but we may also include low concentrations of urea, formaldehyde, distilled water, etc. As soon as the dissociation has taken place, then the prothrombin, cephalin and calcium react to produce thrombin, the thrombin most likely being *prothrombin-Ca-cephalin* with an isolectric point to the alkaline side of neutrality. The combination of the thrombin (+) with the fibrinogen (—) is direct and rapid.

Physiologically, with the clotting of blood in wounds, or in contact with any injured tissue, we do not have to rely on the above dissociating forces to initiate the process. There exists in all tissue cells a protein-cephalin compound, tissue fibrinogen, which has the property of rapid union with blood fibrinogen through calcium. Fibrin formation of this type is entirely independent of aid from all thrombin factors, taking place as well in their entire absence as in their presence. This agent, then, is the natural tissue accelerator of blood clotting, fortifying our defence against loss of blood by being placed in greatest amounts where loss of blood would be most harmful, *i.e.*, the lungs, brain, kidneys, skin and especially in the blood vessel endothelium itself.

It is this tissue agent, then, that begins the dissociation of the plasma complex by removing some of the fibrinogen as fibrin. With the first fibrin formation of this type, some of the thrombin elements are set free, and from there on the two types of clotting are carried on together. However, since the ratio of prothrombin and cephalin to fibrinogen is not 1 : 1, but more nearly 4 : 1, the removal of one molecule of fibrinogen by the tissue fibrinogen thus liberates four molecules of thrombin which in turn join the tissue fibrinogen in removing further fibrinogen. This excess of prothrombin and cephalin over fibrinogen leads to the final fibrin being mainly thrombin fibrin, although that first formed is purely tissue fibrinogen fibrin. Thus we see the tissue factor to be important and essential as a rapid initiator of the clotting process, but the thrombin mechanism to serve as the main clotting force once the process has started. This is well illustrated in hæmophilia, where the tissue fibrinogen clotting is quite normal, but the support from the thrombin side is lacking, and very deficient clotting results. In most cases of hæmorrhages, clinically, the thrombin factors are normal in amount, so that the use of tissue fibrinogen to quicken the starting of the process is logical and practically effective

The wounded tissues, in addition to supplying tissue fibrinogen as an initiator, supply much free cephalin to act as an accelerator of the thrombin production. They, in that way, strengthen both types of clotting.

In addition to the above phases of the clotting process, where the fibrin formation is considered, there exists another almost as important phase, namely, the recovery process. When blood has clotted, that is, when all the fibrinogen has been transformed into fibrin, there is left free about three-quarters of the total thrombin formed. The fate of this reactive and unstable thrombin is the final phase of the process.

The antithrombin present in plasma combined, but free in serum, acts as the stabilising agent in this recovery phase. As serum ages, the thrombin steadily declines in amount, being over 90 per cent. gone usually within an hour at room temperature. This disappearance is brought about in two ways by the antithrombin. The power of the antithrombin lies in its cephalin combining ability. It takes up some of the thrombin directly, through union with its cephalin, and in a second way by constantly taking up any free cephalin from the serum. Since thrombin is unstable and is constantly dissociating off cephalin, the antithrombin, by taking up this cephalin, acts to disintegrate the thrombin and to regenerate prothrombin. The recovery phase, then, sees a partial restoration of the prothrombin and a rapid disappearance of the unstable, active thrombin. An interesting point regarding hæmophilia, again, is that no prothrombin regeneration occurs in such serum, the entire thrombin molecule seeming to be taken up by the antithrombin.

It must be understood that the antithrombin acts in the clotting process only as a stabilising agent in the recovery phase, and takes little or no part in maintaining the fluidity of the circulating blood. The stability of the plasma *in vivo* is due to the lack of dissociation of the clotting factors."

The Conclusions of the Present Writer

There is a fundamental difference between most theories of the fluidity and clotting of blood and that now advocated. Instead of assuming that unshed plasma is a mixture of different colloids, it is regarded as a co-ordinated complex in which the less stable fractions (prothrombin and fibrinogen) are united to the more stable fractions (serum globulin and albumin), and are thus shielded from the disruptive action of calcium ions, which is essential for the inception of blood clotting.

Protection is so afforded against dissolution by small amounts of disturbants, such as are liberated by the natural lysis of blood platelets or may invade the blood-stream when tissue juices are absorbed from wounds.

The complex of plasma colloids possesses only a limited capacity of resisting disturbance. It disintegrates when shed upon any surfaces which it wets and when massive amounts of tissue extracts are rapidly injected into the circulation. By this lysis a complex in equilibrium is replaced by bodies which are susceptible to rapid change. This process is, however, reversible, provided it has not proceeded too far. The formation of reversible gels in the inception of clotting and the development of toxicity in shed plasma prior to its coagulation are thus intelligible.

Many of the difficulties in interpreting blood clotting have arisen from the assumption that all the known facts can be explained by reference to a single process. There are, however, at least three modes of change whereby plasma, after its disruption, can be converted from a sol to a gel. These may be briefly described and illustrated as follows :—

(1) Gels can be produced by the interaction of plasma constituents without the intervention of any other material. This can be demonstrated when bird's plasma, which has been deprived of all formed elements prior to their lysis, is shaken in a sealed tube for half an hour.

Similar gel formation in deplateletised mammalian blood has already been described and occurs when the blood is obtained either at the height of digestion or from persons exhibiting extreme thrombocytopenia. But the gels formed by the interaction of plasma constituents are irretractile, and when seen under the ultramicroscope show hardly any indication of a quasi-crystalline structure. They thus differ from those formed when the *débris*, of either blood platelets or of cells, participates in clotting.

(2) Blood platelets rapidly disintegrate whenever blood is shed. Their *débris* contains protein-phosphatide complexes which flocculate the plasma and assist in the lysis of its complexes, and then participate in the formation of thrombin, which in turn reacts with fibrinogen and produces a gel. Those tissue juices which contain protein-phospholipin complexes react similarly with plasma. In both cases the gels exhibit a quasi-crystalline structure.

(3) Tissue juices unite in varying proportions with the fibrinogen of plasma and produce gels of a similar structure.

So far we are on sure ground, but despite nearly a century of research the precise details of these processes are still partly a matter of conjecture. The hypothesis of Nolf provides a coherent explanation of clotting without the intervention of extra-plasmatic substances, but I am not convinced that thrombin is unsaturated fibrin, and it is not proven that the gels formed are of the same material as the fibrin of normal blood clots. It is also possible that " thrombozyme " is actually a body of extra-plasmatic origin, and is derived from the natural lysis of the formed elements of the blood.

In broad outline, the later developments of the second mode of clotting may be described as follows :—

Part of the prothrombin of plasma is firmly bound, but a fraction is loosely bound. The latter portion unites with the protein-phospholipin complexes of disintegrated platelets and so forms thrombin, but it is possible that a thrombin is produced by the action of calcium ions alone on prothrombin. In view, however, of the ease and rapidity of the reunion of fractions of protein-phospholipin complexes, it appears probable that natural thrombin always contains a phosphatide. It seems established that the active phosphatide is either cephalin or a body closely allied to cephalin.

Thrombin rapidly unites with fibrinogen, and this reaction releases the firmly bound prothrombin, which in turn reacts with the *débris* of platelets and of cells forming thrombin. A progressive increase in the speed of blood clotting is thus produced, which simulates enzyme-action. Besides these reactions, thrombin by its adhesiveness unites micellæ and larger aggregates of fibrin in the manners described by Stübel and by Howell. This action resembles agglutination, but is not identical with that process. Differences in potential between the particles of fibrin and the fluid medium may contribute to these changes.

The modes of union of complexes in the formation of thrombin and of fibrin are not yet fully explained. Attachments by secondary bindings and by mutual salt formation,

in the manner suggested by Sørensen in explanation of the properties of complexes of globulin, together with accessory unions of oppositely charged particles, probably occur.

In the normal clotting of blood in wounds, the second and third modes of gel formation apparently take place simultaneously ; in the later stages of clotting, and when the formation of thrombin is delayed, there is probably also direct union of the disassociated fractions of plasma.

In short, I accept the principal observations recorded in the last twenty-five years, but hold that the current theories are inadequate owing to their authors basing their conclusions on their own observations without giving sufficient attention to the experiments of others.

If the views just advocated fairly represent the truth, a simpler conception of the behaviour of circulating blood follows. Instead of postulating a large number of bodies reacting in unknown ways, the plasma is seen as a unit capable of easily reacting with foreign substances by its unsatisfied side-chains, but returning to a normal condition when the foreign material has been eliminated.

CHAPTER XI

The Arrest of Hæmorrhage

The utilisation of natural processes in the arrest of bleeding—The transfusion and subcutaneous injection of blood—The recognition of abnormal bleeders—The efficacy and standardisation of coagulant hæmostatics—The action of X-rays in the arrest of hæmorrhage—Heliotherapy, vitamin deficiency and hæmorrhage—The risks arising from the use of transfusion and hæmostatics.

APART from surgical treatment, which is outside the scope of this work, modern methods of arresting hæmorrhage are based on the utilisation and augmentation of the natural processes which limit bleeding.

The first natural defence against bleeding is the narrowing of the arteries and veins produced by wounding. The retraction and contraction of the wounded parts by severance of elastic tissues is rapidly followed by a reflex constriction of the arterioles, but these beneficial effects are partly counteracted by dilatation of the capillaries, which causes oozing. Increased vasoconstriction is the principal physiological reaction in the treatment of hæmorrhage by the application of heat or of cold, as in the use of an ice-pack, also in the use of adrenaline and of some astringents. In most hæmorrhagic disorders, including hæmophilic states, the retractile and vasomotor mechanisms are not impaired. These modes of treatment are, therefore, useful in such cases for first-aid purposes, but are usually valueless for the complete arrest of bleeding. The combined local use of adrenaline and of those hæmostatics that increase the coagulability of the blood seems, however, worthy of trial, particularly in epistaxis associated with impaired coagulability of the blood. The local application of a mixture of adrenaline and thromboplastin arrests oozing from the wounds of anæsthetised animals, when these preparations, used separately, are ineffective.

The second natural defence against bleeding is the agglutination of blood platelets in the orifices of broken capillaries and small arterioles. The platelets plug the vessels and so partially arrest bleeding. Those that escape rapidly disintegrate and liberate important participants in blood clotting, also material that promotes vasoconstriction (Stewart, 1914). There is then a physiological basis for the use of the *débris* of blood platelets in the arrest of hæmorrhage, as pointed out by Bordet and Delange (1913), and by Fonio (1913). The vasoconstrictor action is lost, however, in preparations which have been kept for several weeks.

The third and most important defence is the coagulation of blood in wounds. Both thrombin and tissue coagulants participate in this reaction, and extracts of one or other of these materials form the basis of most of the newer hæmostatics. The action of surfaces wetted by blood is also important, since they promote the formation of clots and of coagulants. The efficacy of bandages in the complete arrest of bleeding in normal persons is largely due to such action, as the compression applied is usually insufficient for the prevention of the escape of blood from broken capillaries. For this reason, bandages do not prevent the oozing of blood that is markedly deficient in coagulability.

The value of several of the hæmostatics extracted from blood and tissues has been denied (Hanzlik, 1918, 1, 2; 1919–1920), but it will be shown that these preparations effectively coagulate normal blood. Several factors, however, may modify their efficacy. For the permanent arrest of hæmorrhage the wounded surface must be completely covered with fibrin. A temporary cessation of bleeding follows clotting in the orifices of broken blood vessels, but if the clots are disturbed bleeding recurs. This has long been recognised in the practice of keeping a bleeding limb at rest, but is neglected when styptics are applied either by irrigation or on swabs. The most effective method of using a hæmostatic locally is to apply a bandage soaked in the coagulant.

An important characteristic of shed blood is that the optimum temperature for its clotting is close to that of

the body (Dale and Laidlaw, 1911 ; Pickering and de Souza, 1923; Gibbs, 1924–5). This property of blood plasma provides a natural defence against internal hæmorrhage. The value of hot water as a styptic is due not only to its vasoconstrictor action, but to the provision of a temperature more favourable for blood clotting than that of an exposed wound. In like manner, the hot douches used in post-partum hæmorrhages not only constrict the arterioles, but also hasten the coagulation of the escaping blood. More attention than is usual should be given to the use of heat for the control of accessible bleeding, particularly in epistaxis and in prolonged hæmorrhage from the socket of an extracted tooth. The insertion of a moist plug heated to 40°–42° C. will often stop bleeding when plugs at room temperature fail. The slow impact of a stream of air, similarly heated, on oozing surfaces is often more efficacious than a stream of hot water, as the inhibitory effect of dilution on clotting is avoided and the clots formed are not washed away. When a considerable amount of blood is escaping from damaged arterioles the use of hot air should immediately be followed by the application of a moist bandage heated to 42° C. If bleeding continues, a bandage soaked in one of the thromboplastins mentioned in Table IV. (p. 153) should be applied tightly at body temperature, and the heat maintained by a pack of hot, sterilised plasticine or similar pliant material. An outside bandage of asbestos cloth prevents cooling by radiation. The same table shows that several other hæmostatics are much more effective at 37° C. than at room temperature.

In certain cases that connect normal and hæmorrhagic states, one or both of two defects in the coagulability of the blood may exist, viz., the blood may clot more slowly than is usual at room temperatures (speeds varying from ten to eighteen minutes at 16° C. occur) and the clots formed may be so loose in texture that blood oozes through them. Such bloods often clot promptly and firmly at 40°–42° C., without the administration of any hæmostatic, and this fact may be utilised therapeutically when the bleeding is accessible. A similar condition occurs in some cases that exhibit

all the clinical characteristics of hæmophilia and may be treated similarly (Pickering and Gladstone, 1925, 1).

The fourth defence is the increased coagulability of the blood caused by severe and prolonged bleeding. This is probably due to the passage of tissue juices into the blood-stream and the consequent decrease in the stability of the blood plasma. The apparently spontaneous cessation of severe internal bleeding which sometimes occurs may be due to these causes. A marked increase of the coagulability of the blood follows the internal administration of extracts of natural coagulants and of other disturbants of blood plasma. Great care is necessary, however, in the selection of such bodies. Only those preparations which do not contain intravascular coagulants should be given intravenously, and then only when the hæmorrhage is so severe that there is not sufficient time for the use of subcutaneous or intramuscular injections.

Examples of the treatment of hæmorrhage by direct action on circulating blood are afforded by the use of intravenous injections of coagulen (Ciba) in cases of extreme urgency, by the intravenous or intramuscular injection of sodium citrate (Neuhoff and Hirschfeld, 1922), by the subcutaneous injection of fibrogen (Mills and collaborators, 1923; Mills, 1924, 1926); also by the similar use of hemoplastin, coagulen (Ciba) and thromboplastin-hypodermic (Squibb). The beneficial effects following the oral administration of crude cephalin (Howell, 1916–1917; Hanzlik and Weidenthal, 1919–1920, 2) and of fibrogen (Mills, 1923, 1926), which contains cephalin, are probably due to the passing of the phosphatide from the stomach into the circulation and to its direct action on blood plasma. Further trials of this treatment appear desirable on account of its safety.

The coagulability of the blood is increased during mild anaphylaxis (Widal, 1908). This may occur when the response to sensitisation is no greater than is indicated by a cutaneous reaction. These facts have been utilised in the treatment of obstinate and inaccessible hæmorrhages, particularly those of hæmophilia, by serial injections of horse serum (Emile-Weil, 1919; Vines, 1920; Mills, 1926).

Successful results have also been claimed and corroborated in the treatment of various hæmorrhagic disorders by the production of passive anaphylaxis, sensitised rabbit's serum (the serum anthéma) being used (Dufour and Le Gras, 1914; Dufour and Le Hello, 1921). These reactions are sharply defined and appear to be worthy of greater attention than they have received in this country.

Serial injections of "peptone" have been successfully used in the amelioration of hæmophilia (Nolf and Herry, 1910; Nobécourt and Tixier, 1910; Nolf, 1912). The action of small amounts of "peptone" in provoking hypercoagulability of the blood is, however, more akin to anaphylactoid reactions than to anaphylactic shock (Wells, 1921).

It is stated that fibrinogen obtained from the oxalated blood of normal persons, when deprived of prothrombin and cytozyme by adsorption with tricalcium phosphate, provides a valuable hæmostatic in hæmophilia. The intravascular injection of 20 c.cm. is said to induce a normal speed of blood clotting, which lasts for forty-eight hours, and so permits operations to be performed without excessive bleeding (Frank and Hartmann, 1927.) It seems probable that the exposure of fibrinogen to adsorbents alters its condition so that it behaves like a foreign protein. If this occurs, the reaction *in vivo* is comparable to the production of hypercoagulability of the blood by slight anaphylactoid shock.

It has been suggested that the shock of wounding causes the secretion of adrenaline into the blood-stream, and that the resultant vasoconstriction provides a defence against bleeding. Furthermore, it is stated, by Cannon and his collaborators (1914, 1, 2) that either stimulation of the splanchnic nerves or the intravenous injection of adrenaline shortens the clotting time of blood. The existence of adrenaline in the general circulation is disputed, however, in numerous papers by Stewart and Rogoff (1917–1921), whilst Gley (1921) summarised his work on this subject by stating that adrenaline, in its passage from the suprarenal vein to the heart, is either destroyed, or so diluted that it becomes inactive. A similar conclusion was reached by Zunz and Govaerts (1922). Reviewing this controversy, Sharpey-Schafer (1924) states, however, that despite many assertions to the contrary, there appears little doubt that a discharge of adrenaline may,

under some circumstances, occur in sufficient amount to produce some of the symptoms caused by the intravenous injection of the autocoid. The present writer found that the intravenous injection of sufficient adrenaline to produce well marked vasoconstriction in anæsthetised cats caused a 15–20 per cent decrease of the clotting time of the blood shed immediately after the injection, but blood shed five minutes later clotted no faster than that shed before the injection. More recently, Hirayama (1925) found that both small doses of adrenaline (0 001 mg. per kilo) and large doses (0·05–0·10 mg.) retard the coagulation of blood, whilst medium doses (0·01–0 03 mg.) quicken that change. It appears that such small amounts of adrenaline as may be secreted during shock are insufficient to modify the course of any profuse hæmorrhage.

The action of adrenaline is not cumulative. This fact has been utilised therapeutically in the continuous injection of very weak solutions of the autocoid, which produces vasoconstriction without a marked general rise of blood pressure and so checks bleeding.

Death from cardiac failure arising from intravascular clotting is described by Elliot (1905) as following the intracardiac injection of massive doses of adrenaline in the dog. Dale (1906) noted that any quantity of adrenaline can be injected after the intravascular injection of appropriate amounts of ergot without producing these effects. If Elliot's observations are correct, the clotting must be dependent upon some action which ergot annuls.

A novel view of the natural defence against bleeding is advocated by Stegemann (1924). He maintains that hæmorrhage is controlled primarily by an autonomous side-tracking of the bloodstream, which diverts the blood from the site of bleeding. Be this as it may, both experimental and clinical experience show that if the coagulability of the blood is markedly impaired, any extensive wound may result in fatal hæmorrhage.

The Arrest of Hæmorrhage by the Transfusion and Subcutaneous Injection of Blood

Transfused blood from a donor of the same blood group is, perhaps, the most valuable hæmostatic when bleeding is severe and there is "wound shock." It serves the dual purpose of replacing lost blood and of increasing the speed of blood clotting, even when there is suppressed coagulability. A recent review and an extensive bibliography of this subject has been published by Doan (1927). Attention is also directed to the work of Pauchet and Becart (1924), of E. Weil and Ischwall (1925) and of Morawitz (1926, 2).

In hæmophilia the improvement is only temporary, but may be of sufficient duration to permit of an urgent operation. For example, Minot and Lee (1916) transfused 600 c.cm. of compatible blood into a hæmophilic with a blood coagulation time of 150 minutes. The immediate result was a normal clotting time. Three days later there was a reversion to a hæmophilic state, the clotting time being 60 minutes. Even small amounts of transfused blood may rapidly arrest hæmophilic hæmorrhages (Bernheim, 1917). Other records of this treatment are found in the papers of Hahn (1910), Unger (1917), Bulger (1920) and Fiessly (1925).

Increased coagulability of the blood follows the transfusion of relatively small amounts (10–100 c.cm.) of the citrated blood of normal persons into hæmophilics. Successful results have accrued from this treatment when other hæmostatics have failed (Fiessly, 1924, 1; 1925), but the injection of sodium citrate alone appears almost ineffective (Christie and Gulland, 1927).

The transfusion of a considerable amount of blood arrests hæmorrhage of the new-born. A bibliography of successful cases is given by von Reuss (1921).

Blood transfusion has hardly any permanent effect in purpura hæmorrhagica (Unger, 1919; Doan, 1927), but may be used as a temporary measure until splenectomy can be performed (Whipple, 1926). It has, however, been used with advantage in conditions that exhibit the characteristics of both hæmophilia and purpura hæmorrhagica (Emile-Weil and Ischwall, 1925).

Subcutaneous injections of compatible blood have also been employed successfully in hæmorrhage of the new-born (Schloss and Commissky, 1911; Munro and Eustis, 1922). This treatment is said, by Cruickshank (1924) to be practically specific if the infant is not too severely ex-sanguinated and if its blood exhibits a prolonged clotting-time. From 10–20 c.cm. of blood are injected in the midscapular line. Injections of citrated blood from a suitable donor may be substituted for whole blood (Berghausen, 1918) and when bleeding is severe are employed intravenously (Cruickshank, 1924). A more general use of subcutaneous injections

of whole blood is advocated by Sicard (1922), who employs in adults the patient's own blood instead of that of a donor. From 100–200 c.cm. of blood are obtained by venepuncture, are collected in a vessel coated with sterilised paraffin wax, and then injected into two or three regions of the body. Success is claimed in the arrest of surgical hæmorrhage and that arising from fibromata, hæmorrhoids, hæmophilia and from the administration of the organic compounds of arsenic, such as arsenobenzols.

The mechanisms of these reactions are obscure, but pre-clot changes occur when blood is shed upon a paraffined surface (Novy and de Kruif, 1917) and the anticoagulant action of citrates on circulating blood is rapidly followed by a decrease in the stability of plasma complexes and consequently by hypercoagulability (Pickering and Hewitt, 1925).

The Recognition of Abnormal Bleeders

Excessive bleeding sometimes occurs after an operation, when neither the family history nor superficial appearances give indications of hæmorrhagic tendencies. Except in certain conditions mentioned below, a hæmorrhagic state may be recognised by observing the speed and mode of clotting of a few drops of the individual's blood, obtained by venepuncture in a watch-glass at a temperature of 16°–17° C. Under these conditions, the blood of normal persons clots in 6 to 8 minutes, and the clots formed adhere to the watch-glass, so that it can be inverted without spilling. If the clotting time exceeds ten minutes, or if the clots formed are loose and do not adhere to the watch-glass, a hæmorrhagic tendency is indicated. Careful attention should be given to the appearance of the clots, as a normal clotting time at room temperature may be associated with the formation of loose non-adherent clots and obstinate bleeding from even small wounds. Furthermore, the blood of some profuse bleeders may clot in fractions, whereas normal blood clots completely in a minute after the commencement of coagulation is visible. Similar indications are not usually apparent when the clotting time is observed at body tempera-

ture, in tubes of small calibre, or in films of blood. Most coagulimeters are not suitable, therefore, for this purpose.

This technique does not reveal the presence of purpuric tendencies, which may, however, be recognised by the characteristics mentioned in Chapter XIV. The writer has not had the opportunity of sufficiently testing its value in the recognition of melæna neonatorum, of the hæmorrhagic states arising during sepsis and those following the administration of the arsenobenzols. Cruickshank (1924) emphasises, however, the importance of ascertaining the speed of blood coagulation in hæmorrhage of the new born as a means of distinguishing bleeding arising from congestion from that produced by changes in blood plasma.

The Efficacy and Standardisation of Coagulant Hæmostatics

A summary of the earlier methods of testing the efficacy of coagulant hæmostatics has been given in a recent paper by Pickering and Hemingway (1926, 1). In all these methods, the conditions imposed differ radically from those existing in blood escaping from wounds. It is not surprising, therefore, that the results obtained by different observers are contradictory, the same preparations being described by some as active, and by others as inactive, whilst the clinical evidence is frequently at variance with that deduced from experiments (cf. Hanzlik, 1918–1919; Hanzlik and Weidenthal, 1919–1920, with Hamilton, 1920, and Mills, 1921).

The essential conditions for the accurate standardisation of coagulant hæmostatics may be summarised as follows:— (1) No substance (other than the hæmostatic) which acts either chemically or physically on blood should be present. (2) No substance should be removed from the blood. (3) The blood should be uncontaminated by tissue juices. (4) The blood should remain fluid sufficiently long to observe the effect of the hæmostatic. It is claimed that the following technique satisfies these conditions:

In the standardisation of the local action of hæmostatics, 10–12 c.cm. of blood is shed from an artery of an anæsthetised animal through a paraffined cannula into a paraffined vessel.

By means of paraffined pipettes, 1 c.cm. of blood is immediately transferred to each of a series of paraffined vessels containing 0·25 c.cm. of the hæmostatic to be tested in the dilutions required. Isotonic NaCl is used as a diluent. The speeds of clotting are then observed at 17° C., and at 37·5° C. The efficacy of hæmostatics when administered internally is tested as follows.— 1 c.cm. of blood is shed through a paraffined cannula and is used as a "control." The hæmostatic is then administered and after an appropriate interval, 1 c.cm. of blood is shed from another artery into another paraffined vessel. The time of the completion of clotting (*i.e.*, the formation of a complete gel) is then observed at 17° C. and at 37·5° C. Several bleedings from the same animal should be avoided, as they induce hypercoagulability (Pickering and Hemingway, 1926, 1).

The results obtained by these methods are summarised in the following tables. The blood of cats was used, as it reacts more constantly to coagulants than that of dogs or rabbits. These data are based on 400 experiments.

Similar results are obtainable when human blood, withdrawn by venepuncture, is used. It seems established that the hæmostatics named are efficient coagulants. The older styptics, such as ferric chloride, alum and lead acetate, are unsatisfactory. The precipitates and clots formed by their addition to shed blood are loose and non-adherent.

Experiments with normal blood do not establish the efficacy of a hæmostatic when the blood plasma is abnormal. Preparations which are active in normal conditions may be inactive in hæmorrhagic states. Thus, Fiessly (1924, 1) found that severe hæmophilia was not ameliorated by the use of coagulen (Ciba), hemoplastin and pituitary extract. There is, however, a considerable amount of medical testimony affirming the value of the newer hæmostatics in hæmorrhages of widely different origins. A synopsis of the principal hæmostatics, with references to the experimental and clinical evidence of their value in hæmorrhagic disorders, is given in the Appendix A.

The Action of X-rays in the Arrest of Hæmorrhage

It is established that the coagulability of the blood is markedly increased after exposure of the splenic area to

TABLE IV.—*Showing the Time of Completion of Clotting after the Addition of 0·25 c cm. of various Hæmostatics to 1 c.cm. of Pure Blood at 17° C. and 37·5° C.*

Name of Hæmostatic.	Source of Hæmostatic	Temperature.	Time for Completion of Clotting at Dilutions Stated				
			Full Strength	Half Strength	Quarter Strength	Eighth Strength	Sixteenth Strength
			min sec.	min. sec	min. sec	min sec.	min sec
Coagulen (Ciba)	Blood platelets	17° C	14 12	14 55	12 50	12 10	12 0
		37·5° C.	3 40	3 20	3 40	4 10	3 20
Thromboplastin (Armour)	Brain tissue	17° C	4 0	4 0	4 45	5 35	6 45
		37 5° C	2 0	2 15	1 35	1 40	1 35
Thromboplastin (Ledele)	Brain tissue	17° C	2 5	2 15	2 20	2 50	2 40
		37 5 C.	1 0	1 5	1 0	1 0	1 35
Thromboplastin (Squibb)	Brain tissue	17° C.	3 0	3 15	3 40	3 30	3 45
		37 5° C.	2 10	2 0	2 0	1 55	1 58
Cephalin (Amour), 3% solution	Crude cephalin	17° C	47 0	41 48	26 50	23 0	23 50
		37 5° C.	4 50	5 45	5 45	6 25	5 10
Protogulin	Thrombin	17° C	1 20	1 40	1 10	1 45	2 10
		37·5° C.	0 15	0 45	0 50	1 40	1 10
Hemoplastin (Parke, Davis)	Serum of ox and horse	17° C.	19 30	19 30	16 10	15 0	15 0
		37·5° C	8 10	8 25	4 57	4 48	3 25
Fibrogen (Merrell)	Lung extract	17° C	8 5	8 30	9 17	10 33	13 50
		37·5° C	2 35	3 0	3 30	4 0	4 55

TABLE V.—*Showing the Times of Completion of Clotting of Blood Shed into Paraffined Vessels after the Internal Administration of the Hæmostatics Named*

Name of Hæmostatic.	Amount Administered.	Mode of Administration	Dose in Man Recommended by Makers for General Use.	Time of Completion of Clotting at 17° C.	Time of Completion of Clotting at 37·5° C.
				min. sec.	min sec.
Coagulen (Ciba)	1 25 c cm. of a 3 per cent. solution	Intravenous	10 to 20 c cm of a 3 per cent solution	24 0	10 55
Fibrogen (Merrell)	0 5 c.cm	Intramuscular	3 c cm orally followed by 1 c cm subcutaneously for each 75 lb of body weight	9 20	5 20
Thromboplastin hypodermic (Squibb)	2·5 c.cm.	Intravenous	10 to 20 c cm. every 24 to 48 hours	20 10	8 5
Thromboplastin hypodermic (Squibb)	7 5 c.cm.	Intravenous		19 0	7 20
Hemoplastin (Parke, Davis)	0 25 c.cm.	Intravenous	2 c cm. either intravenously or subcutaneously in severe cases 5 c cm	11 0	8 0
Sodium citrate	5 c cm. of a 10% solution	Intramuscular	30 c cm of 10% solution intramuscularly	17 30	5 40

Note.—Hypercoagulability is evident in a few minutes after intravenous injection (two to three minutes). In intramuscular injection the results are naturally slower In the experiment recorded with fibrogen (Merrell) the blood was shed three hours after injection, in that with sodium citrate one hour after injection.

X-rays (Gramegna, 1905; Stephan, 1920, and later observers). This condition may continue for several hours after the cessation of irradiation and has been utilised in the treatment of inaccessible hæmorrhages and of purpura hæmorrhagica.

Several investigators, notably Saehlof (1921), have shown that hypercoagulability of the blood is produced by the irradiation of other organs, such as the kidneys, the liver, the ovaries and the intestines. It has also been demonstrated that a similar effect follows the irradiation of blood, isolated in the jugular vein by means of ligatures (Fiessly, 1921). Changes in the relationship of the plasma and corpuscles are indicated by the crenation and slight lysis of erythrocytes which are produced by the severe irradiation of human blood *in vitro* (Pickering and Collins, 1923).

Several explanations have been offered of the hypercoagulability of blood in irradiated men and animals. From experiments on shed blood which had been irradiated after oxalation and then re-calcified, Pagniez and his colleagues (1922) conclude that X-rays do not increase the speed of blood clotting *in vitro*: also that the hypercoagulability following irradiation of the spleen is not due to action on the blood itself. It is, however, probable that the stabilisation of the plasma by oxalation prevents the changes caused by X-rays.

Giraud and his co-workers (1921, 1922) suggest that irradiation of the living animal liberates heterologous proteins by cytolysis and that these substances increase the coagulability of the blood. Govaerts and his colleagues (1923) find that severe irradiation (1500–1600 R.) reduces the surface tension of human plasma A physical change in the plasma comparable to that of an anaphylactic shock seems apparent. Definite changes in prothrombin (proserozyme) seem evident from the experiments of La Barre (1925). The splenic area of the dog was irradiated (a dose of 700–750 R. filtered through a 3 mm aluminium screen was used), blood was shed and deprived of fibrinogen by the action of staphylococci. It was found that the prothrombin (proserozyme) left in solution becomes more rapidly active and combines more readily with cytozyme than that of blood which has not been irradiated, but has been subjected to the action of staphylococci. A disturbance of the equilibrum of this fraction of plasma seems indicated. This change may be due to the liberation of cytozyme by irradiation of the spleen. But the observations of Fiessly imply that similar changes occur in the blood itself or that the irradiation liberates cytozyme from the

vascular endothelium. Other changes may follow the irradiation of the splenic region. Zunz and La Barre (1927) found hyperglycæmia and hypercalcæmia accompanied by the passage of an abnormal amount of adrenaline into the circulation. The latter occurrence may temporarily increase the coagulability of the blood.

Recent experiments suggest that blood forced out of the spleen contains bodies that increase the speed of blood clotting McClintock and Magers, 1926). It seems probable that two effects are produced by the irradiation of the splenic area—the liberation of cytozyme and direct disturbance of the equilibrium of the plasma.

Heliotherapy, Vitamin Deficiency and Hæmorrhage

Excessive exposure to sunlight may produce a hæmorrhagic diathesis (Haussman, 1923), which may provoke bleeding from the lungs in tubercular persons. Similar results have been obtained experimentally. The prolonged action of ultra-violet rays on mice causes congestion of the lungs and the escape of red corpuscles by diapedesis (Levi, 1916, 1919; Gassul, 1920). It has also been stated that prolonged exposure to light impairs the coagulability of the blood (Hill, 1925).

From experiments on rats fed on food deficient in the fat-soluble vitamins and kept in light and in darkness, Cramer and his collaborators (1921, 1922, 1923) conclude that sunlight stimulates the production of blood platelets, and that vitamin A participates in the formation of these corpuscles. Cramer, however, reminds me that this work was done before the distinction between vitamins A and D was recognised. It is possible that the latter body may be involved.

Evidence will be presented later which shows that the removal of platelets from the blood-stream is an essential condition in some forms of purpura, and it will be suggested that hæmophilia is due to a defect in the development of the blood. Both of these disorders are refractory to treatment, the latter can be only temporarily ameliorated. It appears advisable to test the response of the body in these conditions to a diet superabundant in the fat-soluble vitamins, administered either alone or in conjunction with moderate exposures of the body to sunlight.

The Risks arising from Transfusion and the use of Hæmostatics

Of the older hæmostatics, both ferric chloride and lead acetate may irritate the bleeding surface (Wilcox, 1923–1924). Calcium chloride is also an irritant and if injected subcutaneously causes sloughing (Grove and Vines, 1921). The intravascular injection of calcium chloride into animals, breathing an atmosphere surcharged with carbon dioxide, produces general intravascular clotting (Wright, 1894). Both intravenous and intramuscular injections of calcium chloride should, therefore, be avoided if there is any considerable increase of carbon dioxide in the blood. Oil of turpentine may cause nephritis, and, except in very small doses (5 minims), is contra-indicated when there is renal inefficiency (Brunton, 1891).

The transfusion of blood may be followed by disagreeable reactions, such as malaise, headache, rise of temperature and urticaria, even when every care has been taken to match the blood of the donor and recipient. Death has followed transfusion when the blood used has satisfied the usual tests for compatibility. Two cases of fatal hæmolysis are recorded by Copher (1923), and Lee (1923) describes the occurrence of anaphylactic phenomena, followed by fatal œdema of the lungs. A summary and bibliography of other fatal cases is given by Baldwin (1925). Such fatalities are not, however, sufficiently common to warrant any hesitation in the use of transfusion in dangerous hæmorrhages.

The condition of the recipient is, nevertheless, of cardinal importance, as it may modify the response to even a well-matched blood. Kordenat and Smithies (1925) point out that transfusion should be made with extreme caution and only in dire emergencies when the subjects exhibit pulmonary, cerebral or peripheral œdema, when the renal output is low, when urine contains albumin, casts or blood; also when the cardiac strength is deficient, with the consequence that there is little resistance to strain.

The causes of the physiological reactions produced by transfusion are still partly obscure. The acute response to the introduction of a considerable amount of incompatible blood into the circulation passes through several phases. The agglutination

of erythrocytes precedes their hæmolysis, and may be followed by a vascular crisis, similar to that produced by anaphylactoid shock. In short, the incompatible blood behaves like a foreign protein. If the patient survives these reactions he may succumb to the increased burden on the kidneys or liver, or possibly from changes in the central nervous system (Kordenat and Smithies, 1925). The same observers believe that as time progresses the recipient's serum (? plasma) develops a lytic action on red corpuscles which is greater than that exhibited when the incompatible blood is introduced.

Hæmatologically, the acute reactions are characterised by delayed clotting and a lower specific gravity of the blood, by falls in the erythrocyte and leucocyte counts, by a striking reduction in the number of platelets, by a lowered polynuclear ratio, and by the appearance of nucleated corpuscles in the blood-stream (Kordenat and Smithies, 1925). It thus appears that not only the plasma but the whole of the hæmatopoietic system is involved.

More difficult is the explanation of the reactions of varying intensity which may follow the transfusion of blood that has satisfied the commonly used tests for compatibility. Some may be due to the fact that bloods which have satisfied these tests may be actually either wholly or partly incompatible The work of Guthrie and Huck (1923) indicates that within the four accepted blood groups are included bloods of diverse sorts, having sufficient in common to secure assignment to the same group, but possessing sufficient differences to manifest incompatibility either by cross-matching or on transfusion. From these observations the same investigators conclude that when several donors are used, it is necessary to re-group the bloods at every transfusion, and in all cases to cross-group the bloods.

The recent observations of Brem and his collaborators (1928) suggest that other factors may produce partial incompatibility of bloods. They noted that a febrile reaction may follow the transfusion of the blood of a donor who is at the height of digestion (particularly when a meat meal has been consumed), and that the blood of the same donor does not produce this effect when obtained during fasting. There is other evidence that the condition of the blood is altered during the height of digestion. It has already been mentioned (on pp. 41 and 42) that the stability of the plasma is so greatly decreased that it rapidly clots after the removal of platelets, and that a similar condition can be produced in the deplateletised plasma of fasting animals by the addition either of proteoses or of certain amino-acids. Furthermore, Czubalkski (1924) found that coagulability of dog's blood is reduced after a " fictitious meal " has been introduced into the stomach. It thus appears probable that some of the unfortunate reactions following the transfusion of apparently compatible

blood may be due to differences in the state of digestion of the donor and recipient. Inquiries should be made on the possible influence of digestion on the capacity of the plasma to agglutinate and lyse corpuscles.

In auto-hæmotherapy, the re-injection of an individual's own blood may cause a severe reaction in persons suffering from prurigo, eczema (either papular or vesicular) and similar diseases. Even a small quantity of re-injected blood (10 c.cm.) may produce a fall in blood pressure, a rise in temperature, thrombocytopenia and leucocytopenia, followed by arthritis. In a case of eczema treated by serial re-injections the disease was aggravated, the blood was rendered incoagulable for nearly three hours, and numerous purpuric patches appeared (Nicolas, Gaté and Duspasquier, 1921, 1923; Monziols and Pouron, 1923).

The risk of fatal intravascular clotting following the intravenous injection of hæmostatics that are prepared from natural coagulants has already been mentioned. The experimental results on animals suggest that the intravenous injection of coagulants should be used only when the rapid arrest of bleeding is essential for the preservation of life, but, so far as I am aware, there is no record of intravascular clotting following the intradermal or intramuscular injection of therapeutic doses of the preparations mentioned in Table V. into normal persons.

A special warning is given by Mills (1924) against the intravenous injection of fibrogen (Merrell), as this extract rapidly produces death by intravascular clotting when injected intravenously. This caution is repeated by the manufacturers. Coagulants derived either from blood or tissues should not be introduced into the blood-stream by any route when there is any liability to thrombosis. Such a tendency occurs in arteriosclerosis, in aneurysm, in phlebitis, in sepsis involving an increase of the fibrinogen of the plasma, and in the puerperium. It may also be present during partial stasis of the blood-stream following wound shock, when severe crushing has caused the absorption of tissue coagulants, in gout and rheumatism accompanied by damage to the vascular endothelium, when vegetative lesions exist

in the vascular system, and according to Emile-Weil and his collaborators (1920, 1922) in certain forms of purpura hæmorrhagica.

On behalf of the American Council of Pharmacy, Hektoen (1924) examined chemically ten proprietary hæmostatics, including coagulose (Parke, Davis), coagulen (Ciba), fibrogen (Merrell), hemagulin (Lily), hemoplastin (Parke and Davis), cephalin (Armour), thromboplastin (Squibb), thromboplastin-hypodermic (Squibb), thromboplastin (Ledele) and thromboplastin solution (Armour). Each of these preparations was found to contain animal protein and may, therefore, cause shock, which may be severe, or even fatal, if the recipient is hypersensitive to protein shock. To this list may be added pituitary extracts, protogulin and all preparations of blood serum. Two cases of fatal shock following the administration of hemoplastin illustrate this danger. In the first case, rather less than 4 c.cm. was injected intramuscularly. The patient collapsed almost immediately and died in a few minutes. It was found subsequently that she suffered from asthma, and that the attacks were elicited by proximity to horses. In the second case, the patient had been sensitised by a previous injection of horse serum (De Lee, 1924). These cases are particularly noteworthy, as this hæmostatic is commonly used without ill effects. Accidents of this kind are of the same order as those occasionally occurring in the use of antitoxic sera, but should not prevent the use of hæmostatic sera, especially when life is endangered by bleeding.

Anaphylactoid phenomena have been produced in guinea-pigs by the intravascular injection of the thromboplastins of Armour and of Squibb, of coagulen (Ciba) and of hemoplastin (Hanzlik, Karsner and Fellerman, 1919–1920). Of these preparations, the thromboplastins used in these experiments are not recommended by their makers for intravenous or intramuscular injection (Thromboplastin-hypodermic (Squibb) was apparently not investigated), whilst the smallest amount of hemoplastin that produced shock in guinea-pigs was fifteen times larger than the maximum dose given in twenty-four hours to human beings (Hamilton, 1920). It may be added that guinea-pigs are peculiarly susceptible to shock.

Fibrogen (Merrell) possesses the power of increasing the anaphylactic response to other protein injections. No other protein should be injected whilst the effect of the fibrogen may continue. If a transfusion is necessary, or if a serum is injected, it should precede the administration of the coagulant. Neglect of this precaution may lead to a severe reaction (Mills, 1924). This warning should be remembered in the internal use of other coagulant hæmostatics. Very little is known of augmentor reactions in "shock" phenomena, but several cases have been brought to my notice in which more or less severe reactions have followed the internal administration of two different hæmostatics during a period of twenty-four to forty-eight hours.

Some degree of shock appears essential, however, for the production of hypercoagulability in circulating blood when the excitatory agent is a protein or a proteose (as in the use of subcutaneous injections of "peptone"). Thus, the newer treatment of either obstinate or inaccessible bleeding aims at producing limited plasma shock (the "choc thromboplastique" of Nolf (1922), or "choc colloidal" of Fiessly, 1922), accompanied by a minimal excitation of general shock. Success depends on the use of a hæmostatic of the required activity and upon the subject not being hypersensitive to its action.

Serious shock has not apparently been reported as following the intramuscular injection of therapeutic amounts of sodium citrate, but severe shock may be produced by the rapid intravenous injection of this salt (Chienisse, 1922). Its administration by any route is, however, inadvisable in purpura and when there is a shortage of blood platelets, as it prolongs the clotting-time of the blood in these conditions (Neuhoff and Hirschfeld, 1922; Higgins and Fisher, 1924). In certain forms of hæmorrhage of the new-born, a pronounced shortage of blood platelets occurs (Morse, 1925), and some cases of later infantile hæmorrhage are apparently akin to purpura (East and collaborators, 1925). The administration of sodium citrate in such cases appears inadvisable.

The risk of excessive shock in hypersensitive subjects

is not limited to use of intravascular or intradermal injections, but may occur when foreign protein has been ingested. In the oral administration of fibrogen, distension of the stomach with iced water is used to prevent the digestion of the hæmostatic. According to Hettwer and Kriz (1925), protein is absorbed under these conditions and may produce anaphylactic phenomena in animals. It appears desirable, therefore, in all cases except those in which bleeding endangers life, to test the reaction of the individual to any hæmostatic containing protein before administering it internally. This may be done by giving, by the route selected, one-fourth of the therapeutic dose. At least half an hour should elapse before giving the full dose, as serial injections of these and similar substances may modify their action in blood plasma (Pickering and Hewitt, 1922, 2 ; 1924, 1). In cases of obstinate hæmorrhages, when the delay necessary for a " cutaneous reaction " is permissible, the use of this test for hypersensitivity is advisable.

CHAPTER XII

The Problem of Thrombosis

The definition and occurrence of thrombosis—The structure of thrombi—The differences in the conditions producing thrombi and extravascular clots—The ætiology of thrombosis—The *rôle* of sepsis in thrombosis—The alleviation of thrombosis.

Thrombosis was defined by Ziegler (1885) as intravascular clotting arising during lifetime. More recent research has, however, shown that this definition is not sufficiently comprehensive. The aggregation either of blood platelets or of polynuclear leucocytes may initiate thrombosis without any apparent deposition of fibrin (Welch, 1887, 1889), and hyaline thrombi may arise in the capillaries from the agglutination of erythrocytes (Flexner, 1902; Boxmeyer, 1903; Pearce, 1904; Loeb and others, 1904; Fåhraeus, 1921). Injury to the walls of blood vessels produces white thrombi, composed solely of blood platelets, when the blood of an animal has been rendered incoagulable by the action of leech extract (Schwalbe, 1907). Moreover, the formation of similar thrombi in the circulating blood of heparinised animals will be described later. Thrombosis can, therefore, be produced, both naturally and experimentally, without intravascular clotting, though most natural thrombi contain blood clots.

The still current descriptions of thrombosis as "necrosis," or as a condition allied to necrosis (Lubarsch, 1924) are premature. The aggregation of blood platelets is probably a physical process, dependent on the condition of the plasma (Roskam, 1922–1923; Aschoff, 1924), and it is not yet established that plasma is a living fluid. Enough has been written to show that a non-committal definition such as the following is desirable: A thrombus is a solid aggregation formed in circulating blood by changes in the blood itself. Thrombosis is the occurrence of such changes.

Thrombi are more common in veins than in arteries. Venous thrombosis is more frequent in the lower limbs than in other parts of the body. It occurs when the only apparent cause is mechanical damage, such as the bruising or crushing of a vein. It will, however, be seen later that these conditions can exist in healthy animals without the formation of thrombi. A list of other conditions that favour thrombosis has already been given (on p. 158). Any one of these states may exist, however, without sufficiently disturbing the condition of the blood to cause either the aggregation of corpuscles or the production of intravascular clots.

Estimates vary respecting the frequency of the occurrence of thrombosis. According to Junge (1912), every hundredth case of gravidity is accompanied by thrombosis, but a much lower percentage is recorded by Bell (1924) when the patients are exercised by movements of the upper limbs. This difference is explicable by the previously mentioned observation (p. 35) that a concentration of natural coagulants, insufficient to clot circulating blood, produces coagula when the blood is at rest in a vein. There are also considerable differences in the estimates of the frequency of post-operative thrombosis. In hospital practice it apparently varies between 1 per cent. and 1·8 per cent. of the number of cases (Franz, 1909; Bondy, 1910; Klein, 1911; Hampton and Wharton, 1920). It may actually be much more common, as de Quervain (1922) suggests that a considerable proportion of the cases of pneumonia following abdominal operations is due to the formation of thrombotic emboli.

There are not sufficient statistics available during the last quinquennium for ascertaining if there has been any substantial change in the frequency of thrombosis in Great Britain and America. But a large increase in the number of deaths from thrombosis and pulmonary embolism is said to have occurred in Germany since 1922. This is explained by Fahr (1927) as due to the more common use of intravenous therapy. Hegeler (1928) denies, however, that the increased mortality has arisen from this treatment or from the use of other remedial measures, such as Rœntgen rays and blood transfusion. He believes that deterioration

of the vascular endothelium and possibly morbid changes in the blood are more common among Germans than formerly. A decision on this question is of considerable importance in relation to the use of intravenous injections in hæmorrhagic disorders.

According to Ribbert (1916, 1, 2), intravascular coagulation is a general occurrence in the onset of death and originates the symptoms which mark a death struggle. This writer states that many clots, commonly regarded as *post-mortem* products, are actually formed during life When a large amount of fibrin is deposited, clotting is said to have occurred some considerable time before death, and the presence of a small amount of fibrin in some clots is explained as due to coagulation having taken place just before death. Marchand (1916) disputes these conclusions and states that the structure of the clots usually found in autopsies is solely determined by gravity. So regular is this action that the position of a body at death may be judged by the proportion of fibrin to corpuscles in a clot. (The attention of students of forensic medicine is directed to this statement.) This controversy appears closed by the observations of Aschoff (1916), who examined a large number of the bodies of soldiers within half an hour after death, and found that intravascular clots are absent, even in cases of death from diseases, in which their appearance is common when later autopsies are made.

The possibility of hereditary factors in thrombosis should not be ignored. Certain hereditary diseases in which the endothelium of the veins may undergo change, such as gout and rheumatism, may provide conditions favourable to thrombosis. Other hereditary factors may also participate. Both Schnitzler (1926) and Hegeler (1928) maintain that certain families are more liable to thrombosis after operations than are normal persons. A more definite indication of genetic influences seems apparent in the occurrence of thrombo-angeitis obliterans among Jews.

The Structure of Thrombi

Thrombi may be classified by their structure. The simplest type of thrombus is composed of blood platelets, with a few entangled leucocytes, and is called a *white thrombus*.

In a short time a layer of fibrinous material, intermingled with leucocytes, is deposited on the platelets. With the ageing of the thrombus the outlines of the corpuscles may disappear, and the whole mass may be transformed into finely granular material (Ziegler, 1885). It is probable that this material is not fibrin. A few erythrocytes are usually entangled in the outer layers, some writers maintaining that they are present in all thrombi (Klemensiewiez, 1907; Benecke, 1913). At a still later stage of development the thrombus may undergo autolysis and be reduced to a fibrinous shell containing fluid (Adami and Nicholls, 1910). Possible liquefaction by bacteria should not be ignored.

When sufficient erythrocytes are entangled in a thrombus, it appears either mottled or laminated. In a *mottled thrombus* the distribution of erythrocytes is irregular, so that in parts it resembles the coagula of shed blood. Distinct stratification, however, is almost invariably present, layers of fibrinous material alternating with deposits of red corpuscles and blood platelets. The lamination may be confined to the region of origin and attachment of the thrombus, when a column of stagnant blood is rapidly and completely clotted in a vein. Such a structure is commonly called an *acute red thrombus* and is hardly distinguishable from either *post-mortem* clots or those of shed blood (Adami and Nicholls, 1910). There is no doubt that except in the laminated portion, these thrombi are typical clots. Identical structures are formed when strings are introduced into the heart of an anæsthetised cat by threading them through the cardiac wall. If the animal is killed three or four minutes after this operation the strings are found coated with blood platelets and filaments of fibrin, with a few entangled red and white corpuscles. In animals killed ten or fifteen minutes later a mottled thrombus is found. When half an hour has elapsed an acute red thrombus is present. It is built upon a central core of platelets, either with or without an intervening laminated area. Typical fibrin can be separated from the clot, and extraction with an 8 per cent. solution of NaCl gives thrombin. The structure of the

thrombus is different, however, when the string is introduced by passing it through the walls of the heart in a fine tube, thus avoiding contact of the string with tissue juices. In its earliest phase the thrombus is almost completely composed of nearly intact platelets. After twenty to thirty minutes have passed the thrombus consists of a core of disintegrating platelets surrounded by laminæ of fibrin, erythrocytes and platelets, with a few leucocytes irregularly disposed. The amount of fibrin is much less than in an acute red thrombus. After one or two hours the original structure is covered with fibrin in which blood corpuscles are embedded, the covering layer being similar to that of a mottled thrombus. It is evident that the development of thrombi is modified by the presence of tissue juices. It also seems clear that the massive clots found in acute red thrombi arise from the ingress of tissue juices into the blood-stream, and that when these coagulants are absent fibrin is not formed prior to the lysis of platelets.

A series of micro-photographs, published by Mason (1924), also illustrates the fact that the different types of thrombi shade one into another. Sections of thrombi produced by the intravenous injection of lung extract show some areas that are indistinguishable from *post-mortem* clots, whilst other areas contain masses of platelets and laminæ of fibrin. More distinct lamination is evident when the thrombus has remained *in situ* for twenty-four hours. A section of a naturally thrombosed vessel in a human lung also shows a similar structure, but the strands and masses of fibrin in the marginal region are rather more apparent. There is then no important structural difference between thrombi produced by the intravascular injection of lung extract and those formed when threads, contaminated with tissue juices, are introduced into the circulation. It is also evident that similar thrombi occur naturally in man.

Thrombi composed wholly of fibrin are said to be formed in the heart and in the pulmonary vessels. Unlike typical white thrombi, they do not adhere to the vascular wall (Welch, 1910). Apparently, this type of thrombus has not been produced experimentally.

The Differences in the Conditions producing Thrombi and Extravascular Clots

Several differences distinguish intravascular clotting from the coagulation of shed blood. The clotting of shed blood spreads rapidly until all the fibrinogen of plasma is converted into fibrin. It has been mentioned that the optimum temperature for this change is at or near that of the body; also that the presence of disintegrating platelets and movement of the blood hastens coagulation *in vitro*. All these conditions exist in circulating blood during the formation of thrombi. Nevertheless, the localisation of clots and their relatively slow increase by deposition of layers of fibrin and corpuscles are the distinctive features of most forms of morbid and post-operative thrombosis.

Although blood remains fluid in a vein isolated from the general circulation (Richardson, 1858, confirmed by many observers), clinicians are agreed that the stasis of blood favours thrombosis, and the decreased resistance of blood immobilised in a vein to clotting by natural coagulants has already been described (p. 35). When a thrombus is forming, blood impinges on masses of disintegrating platelets without losing its fluidity, but the addition either of platelets or of a thrombus to blood *in vitro* hastens its complete clotting. That this difference in behaviour is not due solely to the movement of the blood *in vivo* is shown by the occurrence of localised thrombi, both in the aorta, where the circulation is rapid, and in veins, where it is slow. Particularly striking are those cases of thrombophlebitis in which a vein is almost blocked by a clot, as the blood percolating over the thrombus does not produce coagulation in adjacent veins. When, however, the physical condition of shed blood is preserved nearly unchanged, the mode of formation and localisation of clots resembles that occurring *in vivo*, as is shown by the following experiment:—

The blood of an anæsthetised cat is shed through a paraffined cannula into a paraffined vessel, the bleeding taking place under liquid paraffin, so as to avoid contact of the blood with air. Cotton threads are introduced into the blood by passing them through a fine tube which has been coated with paraffin wax.

The threads thus reach the blood without being in contact with the oil. In a few minutes the threads are covered with clots which are composed of blood platelets, corpuscles and fibrin These clots remain localised for several hours, but slowly increase in size. The localisation is not, however, as permanent as *in vivo*, since the whole of the blood clots in twenty-four to thirty-six hours. The clots found after the strings have been immersed for three or four hours show the structure of a mottled thrombus, except that at the points of attachment to the string both fibrin and erythrocytes are present. There are no areas composed wholly of blood platelets and no indications of lamination. A completely different result is obtained when strings are introduced into blood shed in a paraffined vessel with the blood exposed to the air. The whole of the blood clots in a few minutes.

These observations are explicable on the view that disturbance of the surface conditions of the plasma, sufficient for the dissociation of plasma complexes, is necessary before clotting can occur. Time is required for such changes. In circulating blood most of the plasma passes the thrombus sufficiently rapidly to avoid disintegration, but films of blood adhere to a thrombus and provide the material for its slow increase in size.

The Ætiology of Thrombosis

Much light has been thrown on the formation of thrombi by experiments on living animals, but no single series of experiments explains all the known facts. That local damage to tissues may produce intravascular clotting appears first to have been observed by Samuel (1870), during an investigation of the action of sulphuric acid on the tissues of the rabbit's ear. Two years later, Zahn (1872) noted an accumulation of leucocytes and granules ("Kornchen") in the blood vessels of the mesentery of the frog, when that membrane is injured by crystals of common salt. Blood platelets being unknown at this period, thrombosis was assigned to the accumulation of leucocytes, and the granules described by Zahn were regarded as the *débris* of these corpuscles (Pitres, 1876). Experimenting with the mesentery of dogs and of guinea-pigs, Eberth and Schimmelbusch (1886, 1888) observed that when the blood vessels are first

exposed, the platelets and erythrocytes circulate in the axial portion of the blood-stream, whilst the leucocytes flow closer to the walls of the blood vessels. The slowing of the circulation leads to an accumulation of both leucocytes and platelets in the peripheral blood-stream. When, however, a vessel is injured by ligature, the platelets may adhere to the tunica intima and to one another. These investigators also found that the vascular wall may be injured without causing thrombosis, if the circulation remains sufficiently free to allow the platelets to remain in the axial blood-stream. They thus concluded that the impingement of platelets on irregularities of the vascular wall sufficiently explains the formation of thrombi. These brilliant observations remain unrefuted, but they do not explain the formation of thrombi when microscopic examination reveals no recognisable change in the vascular endothelium. Furthermore, account was not taken of the probable passage of tissue coagulants into the blood-stream when the blood vessels are damaged.

Thrombi can also be produced by the injection of irritants, such as silver nitrate and turpentine, into the perivascular tissue of a rabbit's ear. In the latter case, thrombosis is delayed until œdema appears (Brooks and Crowell, 1908). White thrombi can be produced by the cauterisation of blood vessels (Hanser, 1913), and by the subcutaneous injection of ionisable calcium salts (Bullock and Cramer, 1919).

Prolonged compression of a vein does not cause thrombosis (von Baumgarten, 1903; Rizor, 1903; Brooks and Crowell, 1908). Working with aseptic conditions, Barrett (1924) found that the veins of dogs may be crushed repeatedly without causing thrombosis at the seat of injury. The same investigator fixed sterile threads in the veins of dogs, so that they oscillated in the blood-stream and also released similar threads in the blood-stream, where they moved freely in the circulation for seven weeks. In these experiments the threads must have invaded both the peripheral and axial blood-streams, but thrombosis was not produced. Some cause other than mere impact of blood platelets on

damaged vascular surfaces must be present in the genesis of thrombi.

That alterations in blood plasma provoke thrombosis seems probable from the frequency of thrombi in both puerperal and inflammatory states. Both these conditions are distinguished by a marked excess of fibrinogen in the plasma (see pp. 88, 89) and by a decreased capacity of the plasma to retain its corpuscles in a state of suspension (Linzenmeyer, 1920; Maccabruni, 1921), whilst the decrease in the capability of retaining corpuscles in suspension corresponds with the increase of fibrinogen (Starlinger, 1921; Samson, 1927). Furthermore, Lohr (1921) finds that increases in the rate of sedimentation of corpuscles correspond with the amount of absorption of the disintegration-products of cells. Another factor that favours both the formation of thrombi and decreases the capacity of blood plasma to retain its corpuscles in suspension, is the state of stasis, the speed of sedimentation of corpuscles being increased when blood is confined in a vein (Plass and Rourke, 1927). It thus appears that each of the conditions that favour thrombosis, namely inflammation, the puerperal state, the ingress of tissue juices into the blood and immobility of the blood, also decreases the power of plasma to retain its corpuscles in suspension, and so favours their deposition on the vascular endothelium. Another illustration of the relationship of the suspension stability of the blood with the formation of thrombi is apparent in the observations summarised below :—

The circulating blood of anæsthetised cats and rabbits was rendered temporarily incoagulable by the intravascular injection of heparin. Fine strings were inserted into the ventricles, being passed through the cardiac walls in hollow needles, so as to avoid contact with tissue juices. The needles were withdrawn, leaving the strings in the cavities of the heart. In the cat white thrombi rapidly formed round the strings and a few leucocytes and erythrocytes adhered to the outer surfaces of the thrombi. There was no intravascular clotting. In the rabbit the strings were free from both aggregations of platelets and clots. It was also found that the blood of the cat sediments much more rapidly than that of the rabbit (Pickering and Hemingway, 1926, 2).

Although a close connection between thrombosis and speed of sedimentation of blood corpuscles seems established, it is evident that the changes in plasma which increase the rapidity of sedimentation are insufficient in themselves for the production of thrombi, for as Fåhraeus (1921) remarks, a decreased capacity of the blood to retain its corpuscles in suspension is more common than thrombosis. If, however, an increase in sedimentation is accompanied either by increased agglutinabilty of blood platelets or by the development of adhesiveness in the vascular endothelium, then all the conditions necessary for the inception of thrombi exist.

Several hitherto obscure aspects of thrombosis are explicable when seen from this standpoint. The addition of tissue juices to blood remaining fluid on paraffined surfaces rapidly agglutinates its blood platelets, and a similar conglutination can be produced *in vivo*. When tissues are damaged by wounding, their juices may enter the blood-stream and modify the agglutinability of blood platelets, as well as the capacity of the plasma to hold corpuscles in suspension. When the absorption is insufficient to produce both these results, or if the invading tissue juices are diluted sufficiently rapidly in the blood-stream to render them innocuous, then thrombosis does not occur. The absence of thrombi in most cases of wounding is thus explained. In pregnancy an increased tendency of blood corpuscles to sediment exists long before parturition occurs, but thrombosis is extremely rare in this condition. It is, however, common after parturition. In the latter condition, absorption of tissue juices from uterine lesions may provide the second factor in thrombosis. Primary thrombi may appear several days after a severe operation when sepsis is improbable. This is intelligible as due to slow but cumulative absorption of tissue juices and their passage into the blood-stream in quantities too large to be dealt with by the body.

Some of the rarer forms of thrombosis are also explicable by alterations in the agglutinability of corpuscles, such as the formation of hyaline thrombi in the capillaries by the aggregation of erythrocytes and the production of those

described by Lubarsch (1924) as arising in the capillaries of the spleen, liver and kidney from obstruction by megakaryocytes.

The production of platelet thrombi in pneumonia and during anaphylactic shock is also intelligible. In both these states the agglutinability of the platelets is increased and the coagulability of the blood may be decreased. The former condition favours the appearance of white thrombi, the latter restrains the deposition of fibrin. Furthermore, a great increase in fibrinogen may occur in pneumonia, and also sometimes occurs in anaphylactic shock (Zunz, 1926). In such cases the tendency to thrombosis is increased. It has been mentioned that prolonged venous obstruction causes an increase of fibrinogen in the blood confined in the vein. This may account for the appearance of thrombi in veins which have been obstructed for a considerable time. The increase of fibrinogen may instigate thrombosis.

An interesting explanation of the formation of white thrombi is suggested by Starlinger and Sametnik (1927). They state that blood platelets normally carry an electronegative charge and that the amount of the charge decreases when the concentration of fibrinogen and globulin in the plasma increases. They believe that the decrease in the electrostatic charge enables the platelets to agglutinate if the blood circulates slowly.

In the formation of acute red thrombi, changes in the suspension stability of blood plasma appear to be of minor importance. It has been mentioned that the structure of these thrombi is hardly distinguishable from either *post-mortem* clots or those produced by the intravascular injection of tissue extracts. The rapid ingress of tissue juices into the blood-stream and clotting by their direct union with the fibrinogen of plasma explains the thrombosis. In thrombi which are partly mottled and partly laminated, both factors are apparent—coagulation by direct action of tissue juices and that arising from the agglutination and lysis of blood platelets. It may be assumed that both these kinds of thrombi originate much more rapidly than those which are almost wholly composed of masses of agglutinated platelets.

The recent observations of Van Allen (1927) suggest that the speed of the sedimentation of blood corpuscles is correlated with the changes in plasma which inaugurate clotting. He found that if blood is chilled immediately after collection its corpuscles soon commence to sediment, also that sedimentation continues till clotting commences. When, however, the chilling is postponed for a minute and then instituted, sedimentation does not occur, although the progress of clotting is delayed by cooling. It thus appears probable that the pre-clot changes in blood are an important factor in sedimentation. They may also explain the cessation of the growth of thrombi by the deposition of corpuscles. It has been shown that the blood adjacent to a thrombus suffers changes similar to those which initiate clotting (Pickering and de Souza, 1923), and such changes apparently retard sedimentation.

The influence of contact-action by water-wettable surfaces on the formation of thrombi is illustrated by the recent experiments of Shionoya (1927) and of Johnson (1927). The former observer found that when blood circulates through a system of glass and collodion tubes platelet thrombi are formed before clotting occurs. The latter investigator noted that if the glass tubes are oiled the formation of sufficient fibrin to obstruct them is delayed for three hours.

The rôle of Sepsis in Thrombosis

Much controversy has arisen as to whether sepsis is a prerequisite condition in thrombosis. On the one hand, it is claimed that thrombi occur in the puerperal state when infection is absent (Klein, 1911; Bumm, 1914), and that thrombosis may occur in the brachial vessels after exceptional exertion, without any indications of sepsis (Ivanissevich, 1922). On the other hand, capillary thrombi are common during infection and the larger thrombi often contain hæmolytic organisms. The disintegration-products of erythrocytes contain a coagulant, and it has been suggested that hæmolysis causes thrombosis. Non-hæmolytic organisms have been found in thrombi (Glynn, 1924) and complete hæmolysis can be produced in circulating blood without causing thrombosis. The presence of micro-organisms in a thrombus may have no relation to its formation: the organisms may merely be entangled in the structure, as suggested by Aschoff (1913). According to Bell (1924),

mild sepsis does not cause thrombosis, but the presence of toxins in the circulation is a pre-disposing condition in pulmonary thrombosis. Kretz (cited by Buerger, 1924) states, however, that in 6,511 cases he was unable to find a single instance in which a thrombus was not associated with previous infection, whilst Buerger (1924) concludes that sepsis had played an important part in about half the cases he investigated.

The experimental results summarised below are also inconclusive :—

Jakowski (1889) states that he found thrombi in rabbits and in guinea-pigs, after the intravascular injection of typhoid bacilli, but his results were inconclusive when filtered cultures were used. Talke (1902) claims that the injection of pneumococci into the ear veins of rabbits gives positive results. No thrombi were found by Brookes and Crowell (1908) after virulent cultures of these organisms had been introduced into the circulation of rabbits. Barrett (1924) fixed infected threads into the ear veins of dogs, using *Staphyloccocus aureus and albus*, as well as *Bacillus coli*. After four or five days thrombosis commenced at the points of entry of the threads. The injection of staphylococci into the crushed ear veins of dogs gave, however, negative results.

It is certain that thrombosis can be produced without the intervention of micro-organisms. Inflammation, however, is produced by sepsis. When acute, it increases the amount of fibrinogen in plasma and decreases the suspension stability of the blood. By these changes sepsis may play an important part in thrombosis. In mild infections and when agglutinability of blood platelets is not increased, the disturbance of the blood is insufficient for the formation of thrombi.

In a recent study of the mechanisms of bacterial infection, Wilensky (1926) points out that thrombi may barricade lesions in blood vessels and so prevent the passage of micro-organisms into the blood-stream. A local defensive mechanism against general sepsis is thus provided by thrombosis. In other cases the bacteria grow on the surface of the thrombus and may pass into the blood-stream.

The Alleviation of Thrombosis

Several attempts have been made to prevent thrombosis by experimental methods. Brooks and Crowell (1908) found that thrombi produced by artificial phlebitis are less extensive and are more readily absorbed when the coagulability of the blood is decreased by the oral administration of citric acid. Rabbits were used in these experiments and 2 gms. of citric acid were given daily. When hypercoagulability of the blood was induced by the daily ingestion of calcium lactate, the thrombi were more extensive and less readily absorbed. Marris (1917) recorded seventeen cases of the arrest of thrombosis by the frequent administration of 0·5 gms. of sodium citrate given intravenously. The patients were soldiers who had developed thrombi during enteric infection. These results are not due to reduced coagulability of the blood, as the intravascular injection of moderate amounts of this salt renders the blood hypercoagulable (R. Weil, 1916–1917), whilst the hypocoagulability resulting from large injections rapidly passes off and is followed by decreased coagulation time (Pickering and Hewitt, 1925).

The limitation of thrombosis by a preliminary operation in which either the splenic vein or mesenteric vein is ligatured is claimed by Budde and Kurten (1924). About ten days after this operation a thread was drawn through the portal vein and fastened to the abdominal wall. Scarcely perceptible thrombi were found where the thread had passed. In experiments in which the preliminary operation was omitted all the animals died and extensive thrombi were found in the portal vein. The preliminary laparotomy is believed by these workers to induce the formation of an antigen which limits thrombosis. If aseptic conditions were maintained, the death of all the animals used in the control experiments is surprising, as Wooldridge (1893) produced extensive thrombosis of the portal vein without fatal results. The present writer has confirmed his observations.

The anticoagulant action of heparin has already been

described. It is apparently non-toxic to rabbits and dogs. Mason (1924) found that the intravenous injection of heparin prevents the intravascular clotting produced by the injection of lung extract into the circulation. He suggested that heparin may provide a valuable means of arresting thrombosis in man if the formation of natural thrombi is due to the same causes as intravascular clotting. This appears to be true only when thrombosis arises from the sudden ingress of large amounts of tissue juices into the blood-stream. Furthermore, it has been shown that heparin retains its anticoagulant action *in vivo* for only one or two hours, and that hypercoagulability of the blood follows. The effect of serial intravenous injections of heparin, distributed over several days, has not been investigated. The possible action of intramuscular injections of heparin in the region adjacent to a developing thrombus should also be investigated.

Rowntree (1927) states that heparin mitigates pulmonary thrombosis in man. Although clots are slowly formed they are not sufficiently large to prevent circulation. Details are not given. Similar thrombi are formed when heparinised blood circulates through a system of glass and collodion tubes. A mixture of heparin and $MgSO_4$ is said to prevent thrombosis for one or two hours (Shionoya and collaborators, 1927; Shionoya, 1927).

Intravenous injections of hypertonic NaCl are stated by Silbert (1926) to produce marked improvement in cases of thrombo-angeitis obliterans and, if the disease is not too advanced, to restore the patient to usefulness. One hundred and fifty cubic centimetres of a 5 per cent. solution is given in the first injection, followed by a later injection of 300 c.cm. Beneficial effects are recorded in 46 cases out of 66 treated by this method.

The use of measures, such as exercise, that increase the circulation in any area, where there is a liability to thrombosis, seems justified by both clinical and experimental observations, in cases where movements of the body are not harmful. Immobility of the blood and the consequent tendency of its corpuscles to sediment and of the plasma to clot may thus be avoided. Periodic exercise, first sug-

gested by Pool (1913) as a measure against post-operative thrombosis and as practised by Bell (1924) in gynæcological cases, affords an example of this treatment. To avoid the stasis of blood, Vietor (1925) suggests that in operations positions should be avoided that decrease the circulation by pressure on veins.

The importance of an excess of fibrinogen in the production of thrombi has already been emphasised. Measures for the reduction of any excess to normal limits seem appropriate. A diet composed mainly of carbohydrates and fats markedly reduces the amount of fibrinogen in two or three days.

Very little is known of the influence of medicaments on the sedimentation, agglutination and disintegration of blood corpuscles. Cocaine reduces the agglutinability of blood platelets (Ducceschi, 1915) and heparin prevents their lysis *in vitro* for several hours (Howell, 1927). A promising field of research remains unexplored.

CHAPTER XIII

Menstrual and Puerperal Blood

The coagulability of menstrual blood—The condition of circulating blood during menstruation—The alleged presence of a specific anti-coagulant in menstrual fluid—A note on the arrest of uterine hæmorrhage—Puerperal blood.

MENSTRUAL fluid which has been slowly discharged from the uterus remains unclotted or clots incompletely. When, however, the menstrual flow is rapid, clotting promptly occurs and yields an abundance of fibrin. Several explanations have been offered for the lack of coagulability of the slowly discharged liquid. The earlier writers maintained that an increase of alkalinity, arising from the mixture of menstrual blood with the mucus of the uterus, accounts for the absence of clotting, and this explanation is still current in works on gynæcology. Several facts oppose this conclusion. Small amounts of mucus hasten the clotting of body fluids containing fibrinogen (Birnbaum and Osten, 1906), and the addition of larger quantities, up to 2 per cent., to shed blood only slightly delays coagulation (Whitehouse, 1914). It may be added that a greater amount of alkaline mucus can sometimes be obtained from menstrual fluid containing clots than is obtainable when no clots are visible; also, that the neutralisation of slowly discharged menstrual fluid does not provoke coagulation.

Contamination with vaginal secretions is not the cause of the loss of coagulability. Menstrual liquid obtained without contact with the vagina may remain permanently fluid *in vitro*. Working with the menstrual fluid obtained from twelve cases of hæmatocolpos, Bell (1913–1914) found a shortage of fibrinogen and an absence of thrombin. He concluded that the lack of coagulability in slow-flowing menstrual fluid is due to these deficiencies, and that the

clotting of the rapidly flowing liquid arises from pathological processes. The latter conclusion is disputed by Whitehouse (1914, 1927). He found clots in the uteri of thirty menstruating women who were apparently normal, in twenty-three of whom clots were absent from the vagina. Similar observations were made by Shaw (1925), who also found clots in the uteri of normal women, even at the commencement of menstruation.

Other facts confirm the conclusions of Whitehouse. Blood obtained by puncture of the uterus clots normally (Stickel and Zondek, 1920), and the menstrual discharge of normal women and that collected in the cervix uteri contains thrombin (Kristea and Denk, 1910; Diest, 1912; Strong, 1917). Thrombin is absent from circulating blood, but is present in clotted blood. Its presence in menstrual fluid shows that coagulation has occurred and is of the same type as that which produces blood clots. It appears that the condition of menstrual fluid is determined by the speed of its discharge. When the escape is rapid there is insufficient time for the blood shed from the uterine vessels to clot in the uterus. When the escape is slow, fractional coagulation occurs in the womb and the blood is wholly or partly defibrinated before it is discharged. In addition to the formation of minute clots, it is probable that defibrination takes place by the deposition of a film of fibrin on the walls of the uterus. A similar phenomenon has been observed when blood is slowly shed into the pleural cavity, the defibrination being complete without the formation of clots which are visible to the naked eye (Van Herwerden, 1921, 1922).

Menstrual clots may undergo spontaneous lysis (Whitehouse, 1927). A return to fluidity after the formation of small clots may occur. The incubation of menstrual fluid at body temperature hastens this process. Similar fibrinolysis in slowly clotting blood has already been described, and it has been mentioned that the disintegration of thrombin yields a body which dissolves fibrin. The dissolution of small blood clots in menstrual fluid may thus be explained, without postulating the presence of a specific lysin.

A shortage of thrombin has been observed in menstrual fluid (Kristea and Denk, 1910; Diest, 1912). The adsorption of thrombin by fibrin deposited in the uterus, the autolysis of that coagulant and its "inactivation" by serum probably contribute to this result. The absence of thrombin, described by Bell in menstrual fluid obtained from cases of hæmatocolpos, becomes readily intelligible, as the retention of clotted blood in the uterus subjects it to a temperature which favours autolytic processes.

The Condition of Circulating Blood during Menstruation

The evidence of the development of a specific anticoagulant in unshed blood during menstruation is contradictory. In his monograph on menstruation, Novák (1921) gives two lists of investigators, one of those who observed delayed clotting of systemic blood during the menstrual period, the other of observers who found no such delay. Cantoni (1913) had, however, pointed out that many of the commonly cited observations are unreliable. The bloods compared were often taken from different individuals, and in some cases the temperature at which clotting occurred was not kept constant. The work of Hosaka (1925) throws some light on these discordant results. He compared the speed of clotting of blood in capillary tubes which had been collected from the same women during the menstrual and intermenstrual periods. In some cases there was slightly delayed clotting of the blood shed from the general circulation during menstruation, but in other cases there was no appreciable delay. Similar variations occur in the speed of the clotting of the blood of apparently normal males when coagulation is observed in capillary tubes, but usually cannot be detected when clotting occurs upon a surface which does not adhere to blood, such as paraffin wax. Individual variations in the adhesiveness of the blood during menstruation thus appears more probable than the presence of variable amounts of an anticoagulant.

The onset of menstruation has recently been compared to anaphylactic shock (Carlini, 1924), and it might be inferred that the lack of coagulability of menstrual fluid is similar to the hypocoagulability of the blood during anaphylaxis. It has, however, been mentioned that the coagula-

bility of the blood is increased in mild anaphylaxis, and it seems improbable that any changes occur in the blood during menstruation that are similar to those which suppress blood clotting in severe anaphylactic shock. This conclusion is supported by the observation of Eufinger (1926) that the stability of blood plasma is decreased during menstruation.

The Alleged Presence of a Specific Anticoagulant in Menstrual Fluid

Many observations, particularly those of Novák and Te Linde (1924), demonstrate that disintegration of the endometrium is an essential feature of menstruation. Menstrual fluid differs from blood shed from the systemic circulation as it contains the products of this lysis. It is, therefore, of importance to determine if the uterus or ovaries contain anticoagulants.

Extraction of the female genital organs has yielded variable results. Kristea and Denk (1910) could not obtain any anticoagulant by salt-extraction of the uterus. Schikelé (1912) also failed, but he obtained an anticoagulant from uteri which had been subjected to great pressure. Bell (1913–1914) could not, however, extract any anticoagulant by the latter method. Employing a Buchner press which gave a pressure up to four hundred atmospheres, King (1921) obtained an antithrombin from eight out of fourteen uteri of non-pregnant pigs, and two out of eight pregnant uteri gave similar material. An anticoagulant resembling heparin was obtained from sixteen other uteri, which did not contain antithrombin. But the release of such bodies by the necrosis of the endometrium would not explain the maintenance of the fluidity of the menstrual discharge. The presence of thrombin shows that the suppression of its formation by a heparin-like body, if it occurs, is only partial, and it is also clear that some of the thrombin of menstrual fluid is not neutralised by any anticoagulant. It has already been mentioned that the coagulant action of thrombin is cumulative by releasing bound prothrombin. A minimal quantity thus suffices for the complete clotting of shed blood. The occurrence of both active thrombin and fibrinogen in

menstrual blood which retains its fluidity, therefore, remains unexplained.

Substances which alter the speed of blood coagulation have been extracted from the ovaries by Burckhart-Socin (1915); one called "luteolipoid" hastens clotting, the other named "lipamine" encourages the menstrual flow. It is probable that these bodies are products of the disintegration of the protein-phospholipin complexes of ovarian cells, but it has not been shown that they exist in a free condition in ovarian secretions. More interesting is the statement of Fellner (1924) that blood obtained either from the placenta or from the corpora lutea of guinea-pigs possesses the power of suppressing the coagulation of both systemic and menstrual blood for several days. Human placental blood is said to possess like properties. In explanation of these observations, Fellner suggests that a hormone is produced in the corpus luteum, is discharged into the uterus, and there inhibits blood coagulation. But Allen and Doisy (1927) conclude from a review of the current knowledge of œstral hormones that a specific hormone from the corpus luteum, different from that of the follicle, does not seem to be a necessary causal factor of the menstrual cycle.

A substance which restrains the clotting of blood and is also toxic to plants is said by Macht (1924) to be present in menstrual fluid, and Aschner (1927) maintains that one of the functions of menstruation is the removal of toxins from the body. But Schubert and Steuding (1926) could not find any toxic substance in normal menstrual fluid. The possibility of the absorption of the products of the colloidoclasis of plasma during abnormal menstruation and in *post-partum* hæmorrhage should, however, be investigated. Such an occurrence might well produce auto-intoxication with a febrile reaction, similar to that following the introduction of a foreign protein into the circulation.

A Note on the Arrest of Uterine Hæmorrhage

It appears established that uterine blood possesses all the properties of systemic blood and that if anticoagulants

are liberated during menstruation their influence is of minor importance in the clotting of freshly shed menstrual blood. In short, menstrual blood always clots.

It has been shown that some hæmostatics increase the coagulability of the blood when administered either orally or subcutaneously. Of these preparations, both crude cephalin and the tissue extract called "fibrogen" are particularly active when ingested. These conclusions suggest that their oral administration might prove of value in obstinate uterine hæmorrhages, especially when the bleeding arises from the rupture of capillaries by congestion.

Puerperal Blood

Attention has already been directed to the increase of fibrinogen and to the decrease of the capacity of the plasma to retain its corpuscles in suspension during the puerperal state. The importance of these occurrences in the genesis of thrombi has also been pointed out. Other changes are said to occur. According to Wöhlisch and Bohnen (1924), the citrated blood of pregnant women contains fine, elastic threads of fibrinogen, which adhere to the erythrocytes. It is suggested that the presence of these threads accounts for the rapid sedimentation of the red blood corpuscles in pregnancy. If verified, these observations are of considerable importance, as the presence of threads in the plasma might contribute to the formation of thrombi in the puerperal state. McDonagh (1925) states that directly pregnancy commences, the protein particles of plasma undergo changes which are characteristic of dehydration and lysis. Some of the particles are said to split up before going into solution, others to agglutinate and show a tendency to precipitate. Proof of these statements appears, however, to be lacking.

Changes in the coagulability of the blood have been described by Manuel and Moreira (1926) in both normal and toxæmic pregnancies. In normal cases, the speed of blood clotting in a capillary tube is either unaltered or slightly decreased, the delay in coagulation being more evident when the time of accouchement approaches. Variations in

coagulability are said to continue after normal parturition and may be more marked than those occurring during gestation. In eclamptics the speed of blood clotting may be either decreased or increased, and hypercoagulability seems evident in puerperal toxæmias. The use of a capillary coagulimeter in these experiments throws, however, some doubt on the conclusions reached. The comparatively small differences observed may be due to changes in the adhesiveness and viscosity of the blood and not to alterations in the capacity to clot. These observations should be repeated with blood shed on paraffined surfaces. Under these conditions small variations in the coagulability are patent and are not masked by changes in adhesiveness.

CHAPTER XIV

The Blood of Abnormal Bleeders

The characteristics of hæmophilia and of purpura hæmorrhagica—The classification of hæmorrhagic disorders—The hereditary aspects of abnormal bleeding—The ætiology of hæmophilia—The relationship of hæmophilia to purpura hæmorrhagica—The ætiology of the purpuras and of other hæmorrhagic disorders—The value and significance of splenectomy in purpura hæmorrhagica.

A TENDENCY in certain individuals to bleed abnormally has long been known. It may be manifested either as excessive bleeding from wounds, due to delay in the coagulation of the blood, or by the occurrence of multiple hæmorrhages visible as ecchymoses, whilst the clotting time of the blood does not differ from that of a normal person. The former condition is now known as "hæmophilia," the latter as "purpura hæmorrhagica."

There are other differences which distinguish these disorders. In typical hæmophilia profuse bleeding does not usually occur spontaneously and the coagulability of the blood may vary from time to time in the same individual. The number of blood platelets is either normal (Sahli, 1910; Duke, 1912; Hess, 1916; Gram, 1920) or even slightly above normal (Hynek, 1923). The platelets are, however, less active in promoting clotting than are those of normal blood (Denny and Minot, 1916; Wöhlisch, 1923), but this may apply only to their capacity to clot hæmophilic blood (Fiessly and Fried, 1924). They may disintegrate less rapidly than normal platelets (Davidson and McQuarrie, 1925; Howell, 1926, 1). The blood clots are exceptionally fragile and are thus ineffective in restraining bleeding (Addis, 1910–1911; Nolf, 1913; Hynek, 1923). The duration of bleeding from skin punctures is not excessively long (Hess, 1916; Roskam, 1923); the capillaries are not exceptionally fragile and the morphology of the platelets is normal.

In typical purpura hæmorrhagica the hæmorrhages are apparently spontaneous. There is a marked shortage of blood platelets (Denys, 1887; Duke, 1912; Frank, 1915; Gram, 1920) and deviations from the normal occur in their size and shape (Hess, 1916). Giant platelets are commonly present and may represent masses of platelets which have passed into the circulation before maturity (Piney, 1927). The clots formed in shed blood do not usually contract normally (Hayem and Bensaude, 1901; Nolf, 1913). The frequency and severity of the hæmorrhages vary inversely with the number of platelets and with the capacity of the clots to contract (Morawitz, 1926, 1). The duration of bleeding from skin-punctures is excessively long (Duke, 1915; Hess, 1915; Roskam, 1922–1923, 1927), and the capillaries are more easily ruptured than those of normal persons (Hess, 1916). In the cases which have attracted my attention there were no abnormalities in the fragility and coagulant quality of the platelets.

Another distinction is described by Christie (1927). The second to the seventh drops of either purpuric or normal blood, obtained by skin-puncture, show increased speeds of clotting which remains almost constant. Hæmophilic blood similarly shed by a second bleeding exhibits a decreased coagulation-time, but subsequent bleedings show a rapid return of inhibited coagulability.

Despite these distinctions, a hard and fast line cannot always be drawn between hæmophilia and purpura hæmorrhagica. Cases will be cited later which exhibit some of the characteristics of both disorders, and a case will be described in which hæmophilic and purpuric conditions alternated.

The Classification of Hæmorrhagic Disorders

Although nearly two hundred years ago Werlhoff (1731) recognised the clinical characteristics of purpura hæmorrhagica, it is only in the last few decades that sharp distinctions have been drawn between hæmophilic and purpuric states. In 1911, Bulloch and Fildes defined hæmophilia as "an inherited tendency in males to bleed," and a

few years later, Frank (1915) described purpura as "essentially thrombopenia" (or as "thrombocytopenia"). Both these definitions are not beyond all cavil, but they serve a useful purpose by emphasising the distinctive features of these disorders, and they provide a basis for the classification of many allied conditions. A broad subdivision of hæmorrhagic states is suggested by Emile-Weil and his collaborators (1906–1926). The term "hemogenia" is used to comprise all cases in which a shortage of platelets is associated with irretractile clots and prolonged bleeding from skin-punctures. When these characteristics are present, such apparently diverse conditions as chronic purpura, epistaxis, hæmoptysis and excessive bleeding from the female genital organs are included in this group.

Hæmophilia is recognised as a different condition, in which alteration in the blood plasma is the dominant factor. It is subdivided into hereditary and sporadic forms, the plasma in the latter condition being believed to be in a different state from that existing in the transmitted cases. The same writers use the expression "hemogenic hæmophilia" to comprise those cases in which the characteristics of hæmophilia and purpura are present. These groupings deserve more consideration than has been given them in this country, as they direct attention to the existence of some of the distinctive features of hæmophilia and purpura in conditions not usually associated with these disorders. But they ignore the probable occurrence of different factors within the groups, particularly the changes which cause the localised hæmorrhages in epistaxis, in hæmoptysis and in abnormal bleeding from the female genital organs.

In a recent lecture on hæmorrhagic conditions, Tidy (1926) also recognises the lack of sharpness in the clinical characteristics of purpuric hæmorrhages. It is possible, he states, to trace in different individuals, and sometimes in the same person at different times, every grade between purpura hæmorrhagica, in which the effusions of blood may be large, and those of purpura simplex, in which they are limited to petechiæ. This is consistent with the finding by several observers that each of these disorders is characterised by a

shortage of platelets, by a normal coagulability of the blood, and by a loss of the contractility of blood clots (Grenet, 1915; Nobécourt and Tixier, 1910; Nolf, 1913). There is, then, no distinctive difference, except severity, between these conditions.

Sutherland and Williamson (1925) state, however, that the lack of any increase in the duration of bleeding from skin-puncture is a characteristic of purpura simplex. This may be due to the shortage of platelets being less marked than in the purpura hæmorrhagica.

Adopting the view that the essential occurrence in a hæmorrhagic diathesis is diapedesis, Tidy (1926) suggests the term "angiostaxis" to include the chronic recurrent and hereditary purpuras together with various hitherto unclassified forms of abnormal bleeding, such as local hæmorrhages from the mucous membranes and the ill-defined blood disorders of childhood. In view, however, of the more clearly defined classification of Emile-Weil, the use of this term in a literature already overburdened with nomenclature seems superfluous. In another recent review of this subject, Morawitz (1926, 1) broadly divides purpuric states into "essential thrombopenia" (corresponding with the *Morbus maculosus Werlholfi*) and "anaphylactoid purpura," but he points out that many forms of purpura almost defy classification. This remark also applies to several other forms of abnormal bleeding, such as epistaxis associated with changes in the coagulability of the blood and little or no variation in the number of platelets, the disorders loosely described as "hæmophilia in the female," recurrent epistaxis with a normal blood-picture and coagulability, some forms of melæna neonatorum and the so-called "acquired hæmophilia." In short, until more is known of the biochemistry and biophysics of the blood in hæmorrhagic disorders and of the possible inter-relationship of the metabolism of the blood and tissues all classifications must be regarded as provisional. Subject to these remarks, the following groupings are given, with references to the authorities for their subdivisions.

GROUP 1.—HÆMORRHAGIC CONDITIONS DUE TO DELAYED BLOOD CLOTTING, comprising :—

(A) Hereditary Hæmophilia.—Its principal characteristics have already been described.

(B) Sporadic Hæmophilia.—Cases exhibiting the clinical characteristics of hæmophilia in which hereditary transmission appears improbable have been described by Gettings (cited by Bulloch and Fildes, 1911) and by Davidson and McQuarrie (1925). In the latter case the genealogical inquries extended over three generations, and the biochemical features of hæmophilia were present. Several cases recorded in the literature as "sporadic" are, however, inconclusive, as neither histological nor chemical investigations were made.

(C) Hæmophilia Calcipriva.—The delayed blood clotting is described by Hess (1916) as due to a shortage of calcium in the plasma. Cases which simulate hæmophilia, and are probably due to the lack of ionised calcium, have also been recorded by Emile-Weil (1905, 1906) and by L'Abbé (1909). There is no evidence of hereditary transmission. This extremely rare condition is apparently distinct from hæmophilia, which is not characterised by calcium deficiency (Morawitz and Lossen, 1907).

(D) Pseudo-Hæmophilia.—A number of other cases of more or less spontaneous hæmorrhages associated with inhibited coagulability of the blood have been classed as "acquired hæmophilia," but there is insufficient evidence that hereditary factors are not involved. The condition of the blood in certain cases of syphilis resembles that of hæmophilia, and such cases have also been described as "acquired hæmophilia." For example, Paisseau and his collaborators (1925) observed in a syphilitic man, aged 63, the appearance of a hæmatoma associated with a blood coagulation-time of sixty-five minutes. The blood platelets were not reduced in number, the duration of bleeding from skin-puncture was not prolonged, and the blood clots contracted normally. Injections of neoarsphenamine restored the blood to normality. By this reaction the condition of the blood plasma is distinguished from that of true hæmophilia, which is not alleviated by the administration of arsenical compounds. Cases of so-called hæmophilia connected with syphilis have also been described by Gonzalez-Alvarez (1925). Such cases may be, perhaps, more appropriately described as "pseudo-hæmophilia." But this term, unfortunately, has been used to describe purpuric conditions, such as the hereditary thrombasthenic purpura described as pseudo-hæmophilia by Van der Zande (1923).

(E) Hæmorrhagic Tendencies due to a Shortage of the Fibrinogen of Blood Plasma.—It has already been stated that any extensive destruction of hepatic tissue is followed by a diminution of blood fibrinogen. In extreme cases the blood is rendered incoagulable, with the consequence that any large wound is followed by uncontrollable bleeding. Such a condition may occur in phosphorus poisoning and in acute atrophy of the liver. Nolf (1913) states that the prothrombin of plasma is also destroyed. A similar condition may occur in extensive carcinoma of the liver. Morawitz (1923) points out that hæmorrhages arising from a shortage of fibrinogen may be mistaken for those of hæmophilia, in which there is no such deficiency. The "pseudo-hæmophilia hepatica" of Frank (1925) probably belongs to this group. Also, the so-called hæmophilia produced in the cat by injuring the nerves and lymphatics of the hepatic pedicle (Griffith and Friedman, 1926). In this condition there is a shortage of fibrinogen. A similar deficiency accounts for a human case simulating hæmophilia in which the bile-duct was obstructed (Emile-Weil and Stieffel, 1926).

(F) A Tendency to bleed excessively from Wounds arising from an Increase of Stabilising Material in the Plasma.—Increased thermostability of the blood associated with a capacity of slightly delaying the inception of the clotting of normal blood has been described in cases exhibiting the clinical characterisitics of hæmophilia (Pickering and Gladstone, 1925, 1). Indications of an excess of stabilising material in the blood have also been found in hæmorrhagic disorders associated with grave hepatic disease (Hartmann, 1927). It is possible that abnormal autolysis in the liver may liberate heparin and so delay the inauguration of blood clotting.

GROUP 2.—THE PURPURAS.—Bleeding occurs as effusions into and beneath the skin and in the mucous membranes. There is no marked alteration in the coagulability of the blood. This group may be subdivided as follows :—

(A) Purpura Hæmorrhagica.—The principal features of this disorder have already been mentioned. It may also be stated that its onset is relatively slow and its continuance may be prolonged, the parts commonly affected being the trunk and head (Morawitz, 1926). Cessation of bleeding may occur, however, without any changes in the blood-picture (Piney, 1927) In its atypical forms, one or more of the distinctive features (except the escape of blood from the vessels) may be either absent or nearly absent. Chronic cases occur in which severe thrombocytopenia is associated with a normal duration of bleeding from

skin-punctures, whilst in other cases there is hardly any shortage of platelets and a very prolonged "bleeding-time" (Roskam, 1922–1923). According to Morawitz (1926, 1), purpura hæmorrhagica does not occur in infants, but indications of this condition will be cited later.

(B) Anaphylactoid Purpuras.—These conditions are associated with infections. They are distinguished from purpura hæmorrhagica by the presence of a normal number of blood platelets as well as by normality in the bleeding from skin-punctures and in the contractility of blood clots. Other distinctive features may be summarised as follows:—(1) The development of the disorder is more rapid than in purpura hæmorrhagica (Morawitz, 1926, 1). (2) Both the erythrocytes and vascular endothelium are exceptionally fragile (Silbermann, 1890; Bittorf, 1900). (3) Mild cases show diffuse hæmorrhages close to the hair follicles, which soon disappear by absorption. In chronic cases the hæmorrhages are often urticarial and may be preceded by œdema. In acute cases the bleedings are not limited to the skin, but may be subperiosteal, intramuscular or intravisceral, and there may be lysis of neutrophile leucocytes (Morawitz, 1926, 1). In its most severe form the hæmorrhages spread with alarming rapidity, and this condition was aptly described by the earlier writers as "purpura fulminans" Both "peliosis rheumatica" and "purpura abdominalis" are included in this group by Morawitz (1926, 1). According to Glanzmann (1918), the majority of cases of purpura are of anaphylactoid origin. A similar view is implied by Wendt (1925), but is not generally accepted. The use of the term "anaphylactoid" to describe the disorders should be regarded as provisional, as it is not established that the actual changes correspond with those produced by the experimental introduction of foreign matter into the blood-stream. Many of the purpuras now classed as "anaphylactoid" were formerly described as "toxic," and this grouping is, perhaps, preferable. But some of the purpuras produced by toxic conditions, as in benzol poisoning, exhibit extreme thrombocytopenia. Attention is directed to the isolation of a hæmolytic organism in a case of anaphylactoid purpura (Bullmore and others, 1926). The present writer observed purpuric hæmorrhages in the limbs of a person suffering from protein shock, following the administration (contrary to the makers' instructions) of fibrogen after hemoplastin. There was no thrombocytopenia, and the bleeding and blood-coagulation times were normal. Some of the purpuras complicating pregnancy may possibly fall into this group, since there is often no deviation from normality in platelet count, in bleeding time and in the contractility of blood clots (Rushmore, 1925). According to de Lavergne and Bize (1924), the purpuras

which sometimes follow the administration of antipyrin, neo-arsphenamine, anti-serums and vaccines are anaphylactic, but this classification should also be regarded as tentative, and the term " anaphylactoid " substituted.

(C) Hæmorrhages of Scurvy.—Extravasations of blood of the purpuric type occur in the skin and mucous membranes, the periosteum being affected in severe cases. A distinctive feature is the swelling and bleeding of the gums. There is only a slight alteration in the coagulability of the blood, the platelet count is nearly normal, but the fragility of the capillaries is markedly increased (Hess and Fish, 1914). In experimental scurvy, produced by vitamine deficiency in guinea-pigs, degenerative changes in the vascular endothelium associated with œdema in surrounding tissues are evident. No actual breaches are apparent in the vascular wall, but there is a strong suggestion of the escape of blood corpuscles by diapedesis (Findlay, 1921).

(D) So-called Hæmophilia in the Female.—In many of the cases where a detailed examination of the morphology and biochemistry of the blood has been made, this condition shows one or more of the characteristics of purpura hæmorrhagica. The hæmorrhages often occur as menorrhagia or as epistaxis.

(E) Unclassified Purpuras.—Examples are afforded by " nervous purpuras," including stigmatisation and hæmatodrosis, by the purpuras of acute infectious diseases, by those of chronic disturbances of nutrition and of senility. In the last-named condition definite vascular changes occur (Morawitz, 1926, 1).

GROUP 3.—HÆMORRHAGIC CONDITIONS CONNECTING HÆMOPHILIC AND PURPURIC STATES.—Instances of these disorders will be cited later.

GROUP 4.—EPISTAXIS.—Profuse bleeding occurs from the nose when there is no evidence of trauma. The following forms may be distinguished :—

(*a*) The abnormality appears in both sexes during adolescence and disappears in later life. It is characterised by a normal speed of blood clotting (Lane, 1916).

(*b*) The bleeding occurs in adult females and is associated with a normal coagulability of the blood during the hæmorrhagic periods and suppressed coagulability during the intervening periods. The " blood-picture " is normal (Secco, 1924).

(*c*) The hæmorrhages are associated with a shortage of blood platelets and a normal speed of blood clotting. (Sutherland and Williamson, 1925 ; Sooy and Morse, 1926 ; Giffen, 1927.)

(*d*) The epistaxis is part of a general syndrome in which bleeding occurs from the mouth, uterus and digestive tract. The hæmorrhagic condition may be increased during menstruation and may be associated with thrombocytopenia. Increased fragility of the capillaries is indicated by the production of bleeding by slight pressure on the skin (Hamilton and Waugh, 1924).

(*e*) The only apparent abnormality is an increased fragility of the nasal capillaries.

GROUP 5.—RETINAL HÆMORRHAGES.—An adequate classification of retinal hæmorrhages from the standpoint of hæmatology has not yet been made. Its desirability is illustrated by the occurrence of retinal bleeding in disorders involving different conditions of the blood, such as in hæmophilia (Moore, 1926), during a purpuric syndrome (Hamilton and Waugh, 1924), in adolescents exhibiting hæmorrhagic tendencies with altered coagulability of the blood and as a sequel to thrombosis in the region of the eye.

On the clinical side, Moore (1926) suggests the following groupings: (i.) Hæmorrhages associated with disorders of metabolism, as in renal disease, diabetes and scurvy. (ii.) Those due to diseases of the hæmatopoietic system, in which he includes "atherosclerosis." (iii.) Bleeding produced by congestion following venous obstruction. (iv.) Hæmorrhages due to diseases localised in the eye.

Both Moore (1926) and Duke-Elder (1927) conclude that disfunction of the capillaries occurs commonly in retinal bleeding and is a cause of the escape of blood either by rupture or by diapedesis. These observations suggest a relationship between the condition of the capillaries in such cases and that found in thrombocytopenic purpuras.

In adolescents spontaneous retinal bleeding may be associated with either increased or decreased coagulability of the blood. When the blood is hypercoagulable, improvement may follow the oral administration of citrates; when the coagulability is decreased, calcium lactate may produce beneficial results (Taylor, 1927).

GROUP 6.—HÆMORRHAGES OF THE NEW-BORN.—The bleeding may be intracranial or from the mucous membranes. In true *melæna neonatorum* the intestinal tract is commonly affected. There are at least four types in this group:—

(A) Bleeding from Trauma caused during Birth.—These cases are distinguished by a normal coagulability of the blood (Cruickshank, 1924) and by a normal blood picture.

(B) Hæmorrhages associated with Impaired Coagulability of the Blood.—This condition characterises true *melæna neonatorum*. In severe cases the clotting time may be greatly prolonged (Schloss and Commissky, 1911; Rodda, 1920; Cruickshank, 1924). According to Mills (1926, 1), injury during birth is often a contributory factor.

(C) Hæmorrhages due to Toxic Conditions.—These may be due to infection of the alimentary canal after birth. East and others (1925) cite the case of an infant, aged six weeks, which appears to be a link between anaphylactoid purpura and melæna neonatorum.

(D) Hæmorrhages related to Purpura Hæmorrhagica.—A case in which there was a shortage of platelets is mentioned by Morse (1925). Abnormally prolonged bleeding time, associated with suppressed blood clotting, is described by Rodda (1920), and infantile purpura, with some of the characteristics of hæmophilia, was noted by Payan (1926). A more definite case of thrombocytopenic purpura in a child aged four and a half months is recorded by Greenwald (1927).

The Hereditary Aspects of Abnormal Bleeding

In the Moorish era, Albucasis (1519) described men who, when wounded, suffered uncontrollable bleeding which caused death. Several centuries later, Otto (1803) mentioned a family of hereditary bleeders in which some of the males were affected, but the females, though unaffected, were capable of transmitting this abnormality to their male children. During the next two decades several pedigrees of hæmophilics were published, and from these Nasse (1820) formulated the generalisation associated with his name, stating that only males are subject to hæmophilia and that transmission occurs through apparently normal females. Several important works followed. Grandidier (1855) collated a large amount of information and concluded that hæmophilia occurs in women. Legg (1872) rejected this conclusion, noting that spontaneous hæmorrhages in women are usually associated with sexual disorders and that in such cases bleeding from wounds is not excessive. Neglecting these distinctions, de Bovis (1905) described as "hæmophilic" various forms of immoderate bleeding in women,

including epistaxis and uterine hæmorrhages. A more cautious conclusion was reached by Boyé (1909), who defined hæmophilia as "a hereditary or isolated tendency to either spontaneous or provoked bleeding." This definition he qualified by describing the hereditary disorder as "true hæmophilia" and the non-hereditary cases as "hæmophilic states," which comprise a number of diseases. In an exhaustive monograph, Bulloch and Fildes (1911) analysed earlier records and found that many of the statements pointing to hæmophilia in the female were based on hearsay. They also noted that hæmorrhagic disorders are much less common in the females than in the males of hæmophilic families. In forty-four such families only ten were found to contain females who bled excessively, and these cases only superficially resembled hæmophilia in the male. The so-called exceptions to the generalisation of Nasse, in which hæmophilia is said to be transmitted by a male, were explained as probably due to inter-marriage with a woman who, though apparently normal, was the actual carrier. Subsequent inquiries have largely justified this conclusion. Although Lenz (1923) and other writers describe certain hæmorrhagic conditions in women as examples of hæmophilia, convincing evidence is lacking of any case in which the characteristics of that disorder, as summarised in the opening paragraphs of this chapter, are either present in the female or transmitted by a male.

The existence of hæmophilia in the female is, however, possible, and its rarity could be explained on Mendelian lines. It is generally recognised that hæmophilia is a sex-linked and recessive condition. Piney (1927) points out that the fertilisation of the X chromosome of an ovum carrying the recessive factor by the X chromosome of a hæmophilic sperm would produce an affected female. He suggests that the infrequency of such a union would account for the rarity of female hæmophilics. But it seems equally probable that the offspring of such a tragic mating would not survive till birth.

Two recent writers maintain that a hæmophilic male can transmit the disorder through his daughter to a grandson. Nissé (1927) publishes a pedigree showing three cases. His conclusion illustrates the dubiety of judging solely from clinical evidence. Fortunately he records the speed of blood clotting

in these cases as 4 mins. 30 secs., 6 mins. 30 secs., and 9 mins. respectively, and in one of the affected subjects he found only 87,000 platelets per cmm. of blood. It is patent that the condition of the blood differs from that of a hæmophilic, and in one of the cases thrombocytopenic purpura is indicated. Macklin (1928) reaches her conclusion by deductions from the Mendelian Law and from clinical records which have not been sifted by modern methods. Recent investigations have not, however, lent any support to these suggestions. A renewed inquiry into the Mampel family of hæmophilics affords no evidence of transmission through a male during six generations (Klug, 1926), and a similar lack of evidence is apparent in an American family with a pedigree including eight generations (Davidson and McQuarrie, 1925).

Excessive bleeding, particularly from the genital organs, is more common among the females of hæmophilic families than in families in which the males are normal. In addition to the cases described later, the female bleeders of hæmophilic families may exhibit thrombocytopenia (Bauer and Wehefitz, 1924, 1926), or the tendency to bleed immoderately may be associated with a normal speed of blood clotting, as in the case recorded by Warde (1923). A suppressed or latent hæmophilia, indicated by a slightly increased clotting time of the blood, has been observed in the females of hæmophilic families, but these women do not exhibit typical hæmophilia as it occurs in men (Schloessmann, 1925).

In two families in which the males exhibited mild forms of hæmophilia and in which the transmission conformed to "Nasse's Law," the present writer found that the mothers of the affected males exhibited blood-clotting times which remained within normal limits during a period of three years. In another case the female carrier of severe hæmophilia periodically showed slight variations in the speed of clotting, the times required for complete coagulation at 16° C. varying between six minutes and eleven minutes. Such variations may, however, occur in both the males and females of apparently normal families. Other departures from normality apparently exist in the blood of the mothers of hæmophilic males. Opitz and Lweig (1924) describe decreased permeability of the erythrocytes and state that the

serum contains an excess of calcium, but lacks the trypanocidal power of normal sera.

The absence of typical hæmophilia in the female requires, however, some explanation. From an analysis of the cases collected by Bulloch and Fildes and from those filed in the Eugenic Records Office, Little and Gibbons (1921) suggest that a sex-linked lethal factor eliminates the affected females. A similar conclusion has recently been reached by Mohr (1926) from a study of the mortality in a different group of cases.

The probability of a correlation of "blood groups" with the transmission of hæmophilia has attracted attention. Both Moritsch (1926) and Kubányi (1926) state that in a family they examined, the affected males had the same blood group (II.) as their mother, whilst the males with a different blood group escaped the hæmophilic taint. In Kubányi's case the unaffected males were in Group III. More recently, Kubányi (1927) found only Groups II. and IV. in the survivors of the Mampel family of hæmophilics. Four males are severely affected, and each belongs to Group IV. There is no unaffected male in this group. Renewed investigation on a broader basis is desirable. It might provide the data for prognosis in the sons of hæmophilics long before a hæmorrhagic tendency is apparent.

Other forms of hæmorrhagic disorder may be conveyed from parent to offspring, but the transmission is not sex-linked. Some of these cases simulate hæmophilia, but are distinguished from that disorder by the presence of one or more of the characteristics of purpura hæmorrhagica. For example, Veselý (1923) describes a case in which suppressed coagulability of the blood was associated with thrombocytopenia, as "hæmophilia transmitted by the male." The father, grandfather, and brother were excessive bleeders, as well as a cousin (the son of a maternal aunt). The women were free from the disorder, which, unlike hæmophilia, ceased at the age of twenty. Other families contain members of both sexes who suffer during adolescence from copious and recurrent epistaxis, associated with blood possessing normal coagulability (Lane, 1916). Hereditary

purpuras are mentioned by several writers (Pratt, 1906; Glanzmann, 1918, 1925; Tidy, 1926), but comparatively little attention has been given to the morphology and biochemistry of the blood in these disorders. The transmitted forms are sometimes of the thrombocytopenic type, but Glanzmann states that in nine families, in some of which the purpuric tendency could be detected in three generations, the platelets were not reduced in number, but showed definite malformations.

Familial relationships between chronic purpuras, hæmaturia and epistaxis are also mentioned by Glanzmann, whilst hæmoptysis affecting a family of seven has been noted by Libman and Ottenberg (1913). Giffen (1927) describes hæmorrhagic disease during four generations in which only the maternal line was affected; the manifestations included epistaxis, hæmoptysis and menorrhagia, Hereditary telangiectasis affects both sexes, and is often associated with epistaxis (Davenport, 1912; Williams, 1926; Mekie, 1927). A familial relationship between phlebitis and profuse bleeding is claimed by Emile-Weil and Bloch (1920) and hereditary hæmorrhoids are mentioned by Lahiri (1925),

The Ætiology of Hæmophilia

Pathologists agree that the principal defect in hæmophilia is the suppression of the coagulability of the blood, but no unanimity exists as to the cause of this condition. The first attempt at a biochemical explanation appears to have been made by Sahli (1905, 1910). He found that the same amount of fibrin is obtainable for both hæmophilic and normal blood and suggested that the delayed blood clotting of hæmophilia is due to a shortage of thrombokinase (cytozyme), either in the wounded tissues of the bleeders, or in the formed elements of their blood. Certain facts are consistent with the latter suggestion. Blood obtained by venepuncture from hæmophilics may remain fluid for several hours, but that shed by finger puncture clots much more rapidly (Emile-Weil, 1906; Nolf and Herry, 1909, 1910). Moreover, the addition of tissue extracts to hæmophilic blood hastens its coagulation (Morawitz and Lossen,

1907). A slightly different hypothesis also explains the facts by postulating that the material in plasma which is said to be derived from blood corpuscles, though present in normal quantities, is deficient in activity (Nolf and Herry, 1909, 1910). Recent research has not added any support to these suggestions. Comparative tests with tissue extracts obtained from hæmophilic and normal persons have convinced several observers that in hæmophilia there is neither a shortage nor loss of activity of the tissue juices that promote clotting (Addis, 1911; Minot and Lee, 1916; Lowenburg and Rubenstone, 1918), whilst similar work suggests that this conclusion applies to the participant in coagulation which is derived from the formed elements of the blood (Addis, 1911; Wöhlisch, 1923). Nevertheless, the theory of Sahli is still current (Morawitz, 1926, 1), and hæmophilia has been classed with cystinuria and alcaptonuria as a disease dependent on the lack of an enzyme (Vines, 1920).

It was demonstrated by Addis (1910–1911, 1911) that hæmophilic blood is clotted as rapidly by thrombin as is normal blood, and that the thrombin obtained from the blood of "bleeders" is as active a coagulant as that prepared from normal blood. Other researches show that fibrinogen and thrombin are neither deficient in quantity nor altered in quality (Klinger, 1918; Wöhlisch, 1923) and that calcium is present in normal concentrations (Morawitz and Lossen, 1908; Nolf, 1913; Hess, 1915). Several observers have devised experiments which indicate that the suppression of clotting is not due to the neutralisation of thrombin by antithrombin (Howell, 1914; Hess, 1915; Hurwitz and Lucas, 1916; Klinger, 1918; Fiessly, 1922; Mills, 1926, 3).

The remaining participant in clotting—prothrombin—is also stated by Addis to be present in normal quantities, but several writers have opposed this conclusion (Howell, 1914; Hurwitz and Lucas, 1916; Klinger, 1918; Fiessly and Fried, 1924; Fiessly, 1924). Of these critics, the first and the last have retracted their opinion (Howell and Cekada, 1926; Fiessly, 1925, 3), whilst the experiments of Pickering (1925, 1) and of Frank and Hartmann (1927) sup-

port those of Addis. Having found that all the generally recognised participants in blood clotting are present in normal quantities, Addis (1911) concluded that the defect in hæmophilia is that a longer time is taken for the conversion of prothrombin into thrombin. The cogency of this statement is now generally recognised, and contemporary research is mainly directed to elucidating the cause of the delay. Several hypotheses have been offered. It is suggested that the prothrombin is so altered that its capacity to react normally is impaired (Addis, 1911; Mills, 1926, 3). But the prothrombin separated, by various methods, from hæmophilic blood is as active as that obtained from normal blood (Howell and Cekada, 1926). As an alternative explanation, the presence of a specific substance which prevents change in prothrombin was postulated (Fiessly, 1924, 2, 1925), and Howell (1925) suggested its identity with heparin. Subsequent experiments showed, however, that this explanation is inadequate (Howell, 1926, 1).

In later papers from the same laboratory it is stated that the platelets of hæmophilic blood are more stable than those of normal blood and that the slowness of their disintegration completely accounts for the delayed clotting (Howell and Cekada, 1926; Howell, 1926, 2). In three hæmophilic bloods which I have recently examined the unwashed platelets were more resistant to lysis by contact with water-wettable surfaces than are those of normal blood, and it appears probable that this property is an important factor in hæmophilia. Several facts suggest, however, that it is only one of the conditions which maintain the abnormal fluidity of the blood. The addition of saponin (in concentrations of $\frac{1}{20,000}$ to $\frac{1}{10,000}$) to hæmophilic blood completely disintegrates its platelets, but such blood may remain unclotted for twenty or thirty minutes after the lysis. Beaumont and Dodds (1926) find that hæmophilic corpuscles do not exhibit abnormal resistance to hypotonic saline, and Christie and his collaborators (1927) note that hæmophilic blood after either dilution with water or whipping without defibrination (which destroys platelets) still clots more slowly than normal blood. It may be added that

the blood platelets of hæmophilics which have been washed with isotonic saline rapidly disintegrate on water-wettable surfaces. This suggests that hæmophilic platelets are enclosed in a film of relatively stable plasma, which partially prevents their disintegration. It thus appears probable that the essential abnormality in hæmophilia is an increased stability of the blood plasma.

In the discussion of the stability of normal blood *in vivo*, it has been mentioned that much of the prothrombin is so firmly bound to other plasma protein that it remains stable until released from its union with fibrinogen, and that the actual inception of clotting is due to changes in the loosely bound prothrombin, which is readily dissociated when the blood is shed on water-wettable surfaces. It seems probable that in hæmophilia nearly all the prothrombin is firmly bound, and that the slowness of its dissociation from plasma complexes accounts for the prolonged continuance of the fluidity of shed blood. In support of this suggestion it may be mentioned that the shaking of hæmophilic blood with chloroform produces rapid clotting, and it has been shown that chloroform frees prothrombin from bondage.

Over a century ago, Nasse (1820) suggested that in hæmophilia there is a defect in the growth of the blood. Several facts support this suggestion. At the stage of development when prothrombin and fibrinogen first appear in the lower vertebrates and in the embryos of birds and mammals, the blood remains stable at room temperatures, but is clotted either by raising the temperatures to 30°–40° C., or by the addition of tissue extracts. Hæmophilic bloods behave similarly, but the temperature at which rapid clotting occurs spontaneously may vary between 30°–45° C. It thus appears that the distinctive feature of both hæmophilic blood and that of lower vertebrates and embryos is the exceptional stability of the fibrinogen-prothrombin complex of the plasma. From these facts, it has been suggested that in hæmophilia there is a persistence or a reappearance in adult life of an embryonic condition of the plasma (Pickering and Gladstone, 1925, 1).

The Relationship of Hæmophilia to Purpura Hæmorrhagica

Two family histories of hæmorrhagic disorders, recorded by Hess (1916), illustrate the relationship of these conditions. In the first, two of the males exhibited all the characteristics of hæmophilia, and their female cousin by a maternal aunt was a bleeder of the purpuric type. At the age of eight years she suffered from epistaxis, which occurred almost daily for a year. The extraction of a tooth was followed by hæmorrhage which lasted for a week, but there was no marked suppression of the coagulability of the blood. Later in her life all the characteristics of purpura hæmorrhagica appeared. In the second family a brother and sister were under observation; the male exhibited the distinctive features of hæmophilia, the female those of purpura hæmorrhagica. Attention has already been directed to the finding of a shortage of blood platelets in three female bleeders who were members of a hæmophilic family. In another female bleeder the blood platelets were reduced to 90,000 per c.mm., the blood remained fluid for three days after shedding, whilst oozing continued from a pricked ear for a day (Emile-Weil and Stieffel, 1920). In males, likewise, the characteristics of hæmophilia and purpura hæmorrhagica may co-exist. An instance has already been cited in which the transmission is said to have been from father to son. In another male the shed blood remained fluid for three and a half hours, whilst bleeding from skin-puncture lasted six hours. In this case the number of blood platelets was, however, only slightly reduced, being 280,000 per c.mm. (Koranyi, 1925). Other transitional cases have recently been published. In both a boy and a man the clinical indications of hæmophilia, namely hæmatoma, hæmarthrosis and delayed blood clotting, co-existed with prolonged bleeding from skin-punctures (Emile-Weil and Isch-Wall, 1925, 1), and similar cases are recorded by other writers (Merklen and Woolf, 1925, 1, 2; Comby, 1925; Payan, 1926).

In the following case an alternation of hæmophilic and purpuric states is apparent in a male :—

This individual is stated to have suffered from profuse bleeding from small wounds during childhood. He came under my observation when aged 38 years, 4 months. At that time his blood (obtained by venepuncture) remained fluid on glass at 17° C. for 40–60 minutes, and the number of his blood platelets varied from 340,000–364,000 per c.mm. Six months later his blood-clotting time at 17° C. on glass was 3–4 minutes, his bleeding time from ear puncture was 3–4 minutes, and his platelets numbered 342,000 per c.mm. The periodic variations in the coagulability of the blood found in hæmophilia were thus present. At the age of 40 years petechiæ and ecchymoses appeared, his blood platelets were reduced to 84,000–90,000 per c.mm., and bleeding from ear puncture lasted for 17–24 minutes. At this date his blood-clotting time on glass at 17° C. varied from 11–12 minutes. A year later the external indications of purpura had disappeared, the platelets had increased to 322,000 per c.mm., the bleeding time was 3–4 minutes and blood-clotting time was 17 minutes at 17° C. At the age of 41 years 8 months there was hardly any change in his condition, except that his shed blood remained fluid for 33 minutes at 17° C. A return to a hæmophilic state was thus apparent. One of his brothers was typically hæmophilic. Another brother was described as "normal," but monthly examinations of the coagulability of his blood during a period of two years showed decreased coagulability of the blood on three occasions—the times of clotting at 17° C. being respectively 16 mins. 30 secs., 15 mins., and 13 mins.

The Ætiology of the Purpuras

Blood escapes from the vessels in two distinct ways. A large or sudden hæmorrhage, such as occurs in wounding and in apoplexy, involves a breach in the vascular wall. Gradual bleeding from the capillaries and veins, which may assume alarming proportions, happens without any rent in the blood vessels, and is known as "diapedesis." It is probable that the latter process always occurs in purpura hæmorrhagica, but the sequence of events producing this condition remains partly obscure. A shortage of blood platelets is so common that Denys (1887), and later Frank (1915–1925), suggested that purpura arises from a paucity of these corpuscles. This conclusion has been accepted by the majority of clinicians, and attempts have been made to define the limits of platelet reduction essential for purpuric hæmorrhages. Thus, Duke (1910) places the critical numbers between 40,000 and

75,000 per c.mm.; both Frank (1915) and Morawitz (1923) mention 30,000, but Gram (1920) places the limits between 100,000 and 200,000. In further explanation, Frank (1915) assigns the shortage of platelets to lesions in the bone-marrow, involving the megakaryocytes; but Kanzelson (1916, 1919) maintains that the primary change is an increased activity of the spleen in the destruction of platelets, whilst the bone-marrow normally performs its hæmatopoietic functions. For this reason, he describes any purpuric condition in which there is a shortage of platelets as "thrombolytic purpura."

In support of this conclusion, Kanzelson (1919) points to the occurrence of splenomegaly in purpuric states and suggests that this implies increased splenic activity. He maintains that the great rise in the number of platelets following splenectomy can be attributed only to the removal of the great thrombocytolytic organ. Furthermore, he claims that in thrombocytopenic purpuras there is an excess of megakaryocytes in the bone-marrow and of platelets in the spleen. Histological changes have, however, been described in the megakaryocytes. Seeliger states that in some cases only 4 per cent. are normal (cited by Piney, 1927), and it has been suggested that the spleen removes the abnormal platelets (produced from abnormal megakaryocytes) from the circulation. It is thus possible that the primary changes may occur in these corpuscles. There are other probable explanations. The rate of production of platelets from megakaryocytes may be insufficient to compensate their destruction by the spleen. Changes in the blood plasma may modify the rate of production or quality of the blood. There is some evidence pointing in this direction. Emile-Weil and Stieffel (1925) state that marked differences exist in the quality of human plasmas in relation to hæmatopoietic activity. They state that the plasma of plethoric persons (with erythrocytes 6,000,000 and hæmoglobin 115) contains hæmatopoietic substances. Attempts should be made to extract such bodies, as they might prove of great value in diseases of the blood.

Recent observations show that there are other factors in the purpuric syndrome besides alterations in the rate either of production or of the destruction of blood platelets. It has been demonstrated that the number of platelets may fall below that found in acute purpura hæmorrhagica, even to zero, without producing hæmorrhages (Bedson, 1922,

1924; Roskam, 1922–1923; Ehrlich, 1924). This may occur after splenectomy. Brill and Rosenthal (1923) removed the spleen from a patient exhibiting acute hæmorrhagic thrombocytopenia. Subsequent to this operation, the platelets fell to 1,000 per c.mm., but there was no return of bleeding. The work of Roskam (1922–1923, 1927) and of Bedson (1922, 1924) shows clearly that changes are involved in the peripheral mechanisms that modify bleeding. The former investigators demonstrated that in both natural and experimental purpuras a great reduction in the number of platelets may occur without any considerable prolongation of bleeding from skin punctures. Also, in chronic thrombocytopenic purpura, the "bleeding-time" may not only vary during the course of the disease, but may, at the same time, be different in different parts of the body. For example, in a case of chronic purpura hæmorrhagica, the bleeding-time from the right ear exceeded forty-eight minutes, whilst that from the left ear was only three minutes. The latter investigator found that the injection of anti-platelet serum first damages the vascular endothelium and later produces thrombocytopenia. There follows a leakage of erythrocytes through the vascular wall, which produces effusions of blood by an exaggerated form of diapedesis. The same investigator prepared two different injections. The first damaged the vascular endothelium, the second produced thrombocytopenia. Neither of these preparations provokes purpura when used alone, but when used together they are active in its production. It is thus evident that change in the permeability of the vascular endothelium is an essential occurrence in purpura hæmorrhagica.

According to Bedson (1922), the effect of a shortage of platelets is wholly mechanical. The removal of platelets from the peripheral blood-stream of the capillaries permits the contact of the erythrocytes with the vascular wall and allows their passage through any weak spots in the endothelium. This suggestion does not account for all forms of purpura hæmorrhagica. In some chronic cases, Roskam (1922–1923) found that very prolonged duration of bleeding from skin puncture (48 minutes) may be associated with a relatively large number of platelets

(134,430 per c mm.), whilst in other cases very few platelets (11,020; 11,130, and 15,840) were found when the bleeding time was almost normal. A certain degree of agglutinability of platelets is essential for the arrest of bleeding from the capillaries, and it seems probable that change in the agglutinability of platelets is an important factor in purpura hæmorrhagica. It has been mentioned that the agglutinability of the platelets *in vivo* depends on the condition of the plasma. Changes in the plasma may thus be the decisive factor in the causation of those purpuras in which the platelets are not greatly reduced in number.

Other explanations of the origin of "idiopathic purpura" have been offered. Grenet (1905) produced purpura in the rabbit by ligaturing the hepatic pedicle and then injecting hæmophilic serum into the spinal cord. He concluded that the essential occurrences are intoxication and injuries to the liver and nervous system. Castex (1924) found marked changes in the spinal sympathetic centres in two cases of purpura, which exhibited a reticular distribution. He suggests that such changes are of primary importance in thrombocytopenic purpuras. Emile-Weil (1926) describes the various forms of purpura, including those of toxic or of microbic origin, as the external manifestation of an internal disorder, involving the liver, the circulatory and hæmatopoietic organs, the endocrine glands and the sympathetic nervous system. He regards menorrhagia as an allied condition in which the ovaries are involved. It follows, if these conclusions are correct, that there are close similarities in the syndromes of toxic and thrombocytopenic purpuras. The poor results following the administration of organ extracts (from the thyroid, pituitary and ovary) in purpura hæmorrhagica suggest, however, that if the endocrine glands are involved the changes in them are the results rather than the cause of the purpuric state.

The possibility that alterations in the blood itself are the decisive factor in almost all forms of hæmorrhagic disorder should not be ignored. It is probable that the metabolism of the blood is intimately associated with that of the whole body and that changes in the plasma may find expression in many ways. If the old hypothesis that blood plasma

nourishes tissue is ultimately established, then the multiplicity of changes in hæmorrhagic states becomes intelligible. Defective nutrition might well produce changes in the blood-forming organs, in the peripheral mechanisms which control bleeding and in the condition of the blood platelets.

There are several indications of changes in the plasma in hæmorrhagic states. Alterations in its coagulability, which show changes in its stability, have been described in hæmophilia and allied conditions, in some forms of epistaxis, in retinal hæmorrhages, and in true melæna neonatorum. Nearly all other forms of abnormal bleeding conform either to the thrombocytopenic or to the anaphylactoid type. In typical thrombocytopenic purpura the association of a normal speed of blood clotting with a shortage of platelets points to an abnormal condition of the plasma. Even during fasting, and when platelets are almost absent, coagulability is hardly impaired. This condition is similar to that found in normal blood during the height of digestion, which can be completely deplateletised without losing its capacity to clot. It differs, however, from the normal blood of fasting animals, which, when deplateletised by passage through a clay cell, loses its coagulability. But the latter fluid clots on the addition of certain proteoses and amino-acids (see pp. 41, 42). It thus seems probable that the plasma in thrombocytopenic purpura contains disintegration products which take the place of the *débris* of platelets in clotting, that these bodies are present when digestion is not active and are similar to certain substances which are present in normal plasma during the height of digestion. If these conclusions are correct, it follows that in thrombocytopenic purpura there is an inability to deal normally with the products of digestion; such products accumulate in the plasma, altering its condition and that of the capillaries.

In anaphylactoid purpuras alteration of the plasma by the presence of toxic bodies seems indicated, but careful investigations should be made by similar methods to those employed by Kopaczewski (mentioned in Chapter VIII.) in the study of the plasma during anaphylactic shock.

The Value and Significance of the Effects of Splenectomy in Purpura Hæmorrhagica

The effects of splenectomy in thrombocytopenic purpuras are variable. In the experimental form produced by anti-platelet serum the results are transient. After the operation the platelets increase in number, and there is distinct protection against the hæmorrhage-producing serum, but in a few weeks the animal relapses to its original condition (Bedson, 1924). Similar results arise in some cases of purpura hæmorrhagica. In other cases leucocytosis and an increase in the number of erythrocytes occur without any concomitant increase of platelets (Frank, 1925). But the beneficial effects are frequently prolonged and may possibly be permanent. In Kanzelson's first case, in which the spleen was removed in 1916, there has been no recurrence of hæmorrhagic symptoms (Whipple, 1926). In the majority of fifty cases, noted by Farley (1925), the value of splenectomy is apparent. Whipple (1926) mentions several instances in which there has been no relapse for a year or longer. Of sixty-one cases which he followed up, fifty-one gave good results. Improvement was, however, found only in the chronic cases, and was most marked when there was hypertrophy of the spleen. It is noteworthy that Whipple regards splenectomy as hazardous in acute cases.

It has been recently suggested that the whole of the reticulo-endothelial system may be involved in thrombocytopenic purpuras (Brill and Rosenthal, 1923; Emile-Weil, 1926; Jedlička and Altschuller, 1926). This hypothesis explains the variable results following splenectomy. For, as Whipple (1926) remarks, if over-activity in the destruction of platelets is limited to the spleen its removal should give consistently good results, but if the whole reticulo-endothelial system is involved, extirpation of the spleen would remove only a part of the platelet-destroying apparatus.

The recent observations of Rosenthal (1928) and of Giffen (1928) throw additional light upon the different effects which may follow the removal of the spleen in purpura hæmorrhagica and emphasise the conclusion reached earlier in the chapter that this disorder is not always sharply

defined. This work also shows that clinical observations, even when aided by an examination of the "blood picture," may not reveal important departures from the typical condition.

In thrombocytopenic purpuras, the effusions of blood may be either the predominating occurrence or merely symptomatic of a more general disturbance. Distinct differences may appear in both the blood picture and in the behaviour of the blood in clotting. (1) An extreme shortage of platelets (below 10,000 per c.mm.) may be associated with the formation of irretractile clots. (2) When the thrombocytopenia is not extreme, clot-retraction may be slight or even absent. (3) The clots may be irretractile when the platelets are much more abundant (20,000–90,000). Both delayed and normal blood clotting occur when the clots are either retractile or irretractile, when the platelets are greatly reduced in number and when they are relatively abundant (Rosenthal, 1928).

In unusual forms of hæmorrhagic disorder, Giffen (1928) describes sharply defined differences in the blood picture, in the platelet count, in the speed of formation of thrombin and of complete clotting ; also in the extent of the contraction of clots. In some of these cases the superficial characteristics of purpura hæmorrhagica are so well marked that they might readily be described as typical cases if attention was given only to the clinical manifestations and blood picture.

The cases recorded by Giffen (1928) show, however, that the extirpation of the spleen may sometimes be followed by the cessation of hæmorrhage and by a general improvement in bodily condition when only some of the characteristics of purpura hæmorrhagica are present. But purpuras not manifesting thrombocytopenia are apparently not benefited by the removal of the spleen, even when all the other features of purpura hæmorrhagica are present.

APPENDIX A

A SYNOPSIS OF THE NEWER HÆMOSTATICS

In selecting a hæmostatic it is essential to have a knowledge of the state of the blood, particularly of its stability, as indicated by its coagulability and the number of platelets present. Unfortunately, in the majority of medical reports on the use of hæmostatics a record of these factors is not given. It is frequently stated that a particular hæmorrhage has been completely arrested by the use of one or other of the proprietary hæmostatics, as in the many accounts of the treatment of epistaxis, of hæmatemesis and of hæmoptysis. Such hæmorrhages may arise from totally different conditions, and evidence of efficacy in one case may not be applicable to another. Furthermore, even obstinate hæmorrhages may cease spontaneously from causes not yet fully known, a probable factor being the absorption of coagulant tissue juices from the lesion. Such an occurrence might easily be recorded as an example of successful treatment if a hæmostatic had been used. Nevertheless, the amount of evidence of the efficacy of the newer preparations in a wide range of hæmorrhages is now so considerable that it warrants careful attention.

It should, however, be noted that some forms of abnormal bleeding are almost completely resistant to the newer hæmostatics. Examples are provided by the severer forms of hæmophilia and by a case of epistaxis (recorded by Giffen, 1928) in which all the characteristics of purpura hæmorrhagica were present except thrombocytopenia. Bleeding by diapedesis is usually refractory to hæmostatics, but some hæmorrhages which form part of a purpuric syndrome may be arrested by appropriate subcutaneous or intramuscular medication.

CEPHALIN

The use of crude cephalin as a coagulant hæmostatic is due to the work of Howell (1912–1913, 1916–1917). It was first employed therapeutically by Hirschfelder (1915), who applied it on gauze or as a wash in surgical bleeding. Cecil (1917) used gauze and catheters coated with cephalin in thirty-four cases of hæmorrhage following perineal prostatectomy. In some cases bleeding ceased in four or six hours, in several others the drainage fluid was free from blood on the following morning, in four only were there indications of bleeding after forty-eight hours. The cessation of obstinate bleeding following the oral administration of cephalin has been described by Howell (1916–1917) and by Hanzlik and Weidenthal (1919–1920, 2). In the first case 100 c.cm. of 0·1 per cent. solution of Howell's cephalin (extracted from brain substance) was given twice daily to a congenital hæmophilic. In the second case, 4 grams of cephalin was given to a person suffering from obstinate intestinal bleeding, said to be hæmophilic. In both cases the bleeding promptly ceased. Bastedo (1919) advises oral administration in hæmatemesis. Further trials of this treatment appear desirable on account of its safety. Cephalin has been used intravenously in severe pulmonary hæmorrhage, but this procedure involves grave danger from intravascular clotting.

COAGULEN—(CIBA)

This hæmostatic is a preparation of the blood platelets of the ox, with a small amount of lactose added to facilitate solution. The makers advise its use as follows :—Locally a 3–5 per cent. solution, or on gauze ; subcutaneously 20 c.cm of a 3 per cent. solution once or twice daily (in slight cases, 1·5 c.cm. of a 3 per cent. solution several times daily). Intravenous injections are only advised in extreme cases, with the caution that they should be discontinued if there are signs of respiratory or cardiac failure. By the mouth, 5 grams dissolved in 200 c.cm. of distilled water is suggested, divided into equal doses during twenty-four hours. In rectal hæmorrhages, 1 to 2 grams in 50 c.cm. of distilled water are given as an enema. In dental bleeding, local application and the injection of 3–6 c.cm. of a 3 per cent. solution into the gums is advised.

Favourable medical reports led to the inclusion of this preparation among the hæmostatics mentioned in the 1915 edition of "New and Non-official Remedies," but it was excluded from the 1922 edition owing to a very unfavourable report by Hanzlik (1922). In the opinion of the present writer, this arose from faulty experimentation (see p. 151).

Attention is directed to the following reports of successful use in medical practice. The arrest of bleeding in purpura hæmorrhagica is recorded by Benoit (1924), and in a condition allied to that disorder in which the coagulability of the blood is impaired (Merklen and Wolf, 1926). The latter statement deserves careful consideration, as its authors have paid special attention to this hæmorrhagic condition.

A well-recorded case of the use of Coagulen (Ciba) in severe hæmoptysis, arising from pulmonary tuberculosis, is given by Morland (1925). The subcutaneous injection of 5 c.cm. arrested the bleeding for half an hour. The hæmorrhage then recurred, and 5 c.cm. was injected into the cavity of the lung through an intercostal space. Bleeding ceased suddenly, and after reappearing in lesser amount gradually stopped. The treatment appears to have been a complete success. Irvine (1925) reports favourably on the use of this hæmostatic in intestinal bleeding during typhoid fever, and Papadopoulos (1925) used it successfully in the control of hæmorrhage after tonsillectomy.

Negative results were obtained by Fiessly (1924, 1) in severe hæmophilia, but Morawitz (1926, 1) cites its use in that disorder by Derselbe (1913), by Frank (1915) and by Fonio (1916–1917). Preparations of blood platelets have been given intravenously, intramuscularly and by the mouth in the treatment of gastric hæmorrhage (Krecke, 1925).

EUPHYLLIN

This preparation is composed of primary and secondary theophylline ethylendiamine in equal proportions. Its local use in hæmophilic hæmorrhages is mentioned by Morawitz (1926, 1).

FIBROGEN

This hæmostatic is an extract of lung tissue prepared by the method of Mills (1921, 1, 2). It is administered either subcutaneously or by the mouth and should not be given intravenously, as it is an intravascular coagulant. The dosage recommended by the makers is given in Table V. (p. 153). As many as five injections of 1 c.cm. have been given during an afternoon in an adult, while a 30-pound boy has received 1 c.cm. twice daily, with no effect except the cessation of hæmorrhage. Blood pressure, pulse rate and the speed of respiration are said to remain unchanged, but there is some pain at the site of the injection. About one or two hours are required for the hæmostatic effect to reach a maximum.

When given by the mouth in amounts of 4–5 c.cm., followed by a glass of iced water, the effect is more rapid. Mills (1924) mentions

its successful use in hæmorrhages of pulmonary tuberculosis and those of typhoid fever, in epistaxis, in bleeding from gastric ulcers, also in surgical hæmorrhages, particularly those following tonsillectomy.

The complete arrest of obstinate bleeding by the oral administration of fibrogen is described by Dickey (1926) in two cases in which the blood examinations and bleeding time from skin puncture showed the characteristics of hæmophilia, although there was no evidence of hereditary transmission. In the first case four doses of 1·5 c.cm. sufficed in an infant suffering from continuous bleeding after circumcision and from urethral hæmorrhage. In the second case (a boy aged twelve years, exhibiting a hæmatoma and a blood-clotting time of 25 minutes), the administration of 3 c.cm. was followed by the cessation of bleeding and the absorption of the effusion. In both cases there was a marked increase in the speed of blood clotting. In a few days marked inhibitions of coagulability followed, and the administration of fibrogen did not again increase the rapidity of clotting. But there was no return of bleeding whilst the patients remained under observation.

GELATIN

According to Nohl (1910), the treatment of hæmorrhage of the new-born by the subcutaneous injection with sterilised gelatin reduces the mortality from 50 per cent. to 5·5 per cent. Von Reuss (1921) advises enemas of 200 c.cm. of Merck's preparation or the subcutaneous injection of 10–20 c.cm., the latter treatment to be repeated on the same or the next day if bleeding continues. Wetterdal (1926) has used blood transfusion with subcutaneous injections of gelatin in melæna neonatorum. One to seven injections were given. Twenty-seven infants died out of sixty-seven so treated, but many of the deaths are attributed to the injections being used too late.

Subcutaneous injections of one or two ampoules of Merck's sterile gelatin are mentioned by Morawitz (1926, 1) as advantageous in hæmophilia.

HEMAGULIN

This hæmostatic is an extract of brain tissue and possesses similar properties to the thromboplastins.

HEMOPLASTIN

Hemoplastin is prepared from the serum of horses and oxen by a secret process based on the work of Lapenta (1919). Very wide claims have been made for its efficacy in the treatment of hæmorrhagic conditions, but the records in medical journals do

not afford much assistance in appraising its value. A few examples of the evidence will illustrate the position. Brown (1922) states that the injection of 2 c.cm. of hemoplastin is of value as a prophylactic against hæmorrhage after tonsillectomy. Nash (1923) records that the subcutaneous injection of 2 c.cm. was followed by the cessation of bleeding in an infant suffering from melæna neonatorum, but he makes no mention that his diagnosis was confirmed by an examination of the blood. Yearsley (1925) injected 1 c.cm. of hemoplastin after an operation on septic tonsils. The bleeding stopped, but recurred on the next day. Howkins (1925) advises the oral administration of calcium lactate and the giving of two injections of 2 c cm. of hemoplastin in dental hæmorrhage. He adds that plugs soaked in oil of turpentine should be used if the bleeding continues. Burke (1925) mentions the arrest of hæmophilic bleeding by the use of hemoplastin in conjunction with the intramuscular injection and transfusion of blood as well as the local application of adrenaline. Lahiri (1925) claims the complete arrest of severe uterine hæmorrhage by three subcutaneous injections of hemoplastin (each of 2 c.cm.), and like success in the treatment of prolonged bleeding from hæmorrhoids by the use of four injections. Fiessly (1924, 1), as well as Christie and Gulland (1927), could not arrest hæmophilic bleeding by the use of hemoplastin.

Blood examinations were made by the present writer, both before and after the administration of hemoplastin, in the case of a boy, which was described by the physician in charge as "hæmophilia." The grosser symptoms were a large hæmatoma in a knee joint and bleeding from the gums, which had continued for three weeks. The administration of six subcutaneous injections of hemoplastin (each of 2 c.cm.) at intervals of 24 hours was followed by cessation of bleeding and later by absorption of the effusion. Before the administration of the serum the boy's blood showed a clotting time at 16° C. of 15 minutes; his bleeding times from ear punctures were, respectively, from the right ear 20 minutes, from the left ear 28 minutes. A platelet count showed 100,000 per c.mm. After the cessation of hæmorrhage, the blood-clotting time at 16° was 9 minutes 30 seconds, the bleeding times for ear punctures were almost unchanged, and the platelets numbered 100,300 per c.mm. This case thus closely resembled those now classed as "hæmophilic hemogenia." It is noteworthy that the bleeding ceased whilst the indications of a hæmorrhagic tendency continued.

OIL OF TURPENTINE

A new use of oil of turpentine is advocated by Steadman (1923), who employs pledgets soaked in the oil in dental surgery. He

mentions that in fifty cases this treatment was invariably successful.

PARATHYROID EXTRACT

An extract of the parathyroid gland was used by Vines (1922) in the treatment of hæmatemesis arising from gastric ulcers which had frequently occurred during a period of seventeen years. The daily oral administration of $\frac{1}{10}$ grain of the dried gland for a month was followed by the cessation of bleeding.

The recent work of Collip, alone and in collaboration (1925–1927), has given a new impetus to research. It was shown that the administration of the parathyroid hormone effectively and consistently increases the amount of calcium in the blood, the concentration in serum rising from 10·5 mg. to 20 mg. per 100 c.cm. or even to a greater extent. The known *rôle* of calcium in blood clotting naturally suggested the re-investigation of parathyroid extract as a hæmostatic. Cantarow and his collaborators (1926) found a definite increase in the coagulability of the blood four hours after the injection of Collip's extract. The hypercoagulability reached a maximum in 10–15 hours and gradually passed off, a return to normal coagulability being reached in 20–30 hours. The same group of investigators used subcutaneous injections of 10–15 units during a period of 30–36 hours (in a few cases 15–20 units were given during 20–24 hours) in the treatment of a wide range of hæmorrhages, including bleeding from pulmonary tuberculosis, hæmorrhages from the gastro-intestinal and urinary tracts, in jaundice, in various unspecified conditions in which blood clotting is delayed; also in post-operative hæmorrhage. Of 364 patients treated, 347 are said to have ceased bleeding (Gordon and collaborators, 1926; Gordon and Cantarow, 1927).

The action of the parathyroid hormone has not been tested either in hereditary hæmophilia or in hæmophilia calcipriva. In the latter condition it may be of value, as the defect in the coagulability of the blood arises from a shortage of calcium ions.

Overdosage if long continued in animals produces extreme hypercalcæmia and secondary changes which may culminate in death (Collip, 1927). Unfavourable results also follow overdosage in man, and inimical effects are produced in puerperal hæmorrhages and in melæna neonatorum, even when the dosage is not excessive (Gordon and Cantarow, 1927). In all cases the continuance of administration should be controlled by determinations of the calcium in the patient's serum. According to McCann (1927), a concentration of 15 mg. per 100 c.cm. indicates danger.

PECTEN

The hæmostatic action of pecten (prepared from gentian) is described by Violle and Saint-Rat (1924) and later by Violle (1925), who advise its use in both normal persons and those suffering from hæmorrhagic tendencies. Fiessly (1925) investigated its action on the circulating blood of rabbits, dogs and man, employing a 1 per cent. solution, rendered isotonic to blood by the addition of 0·5 per cent. $CaCl_2$ and 0·75 per cent. NaCl. (The solution was sterilised at 120° C.) In the rabbit, the intravenous injection of 0·5 c.cm into animals of average weight reduces the clotting time of shed blood by one-half. In the dog, the injection of 1–2 c.cm. for each kilogramme of the animal's weight causes the blood to remain fluid after shedding for several hours. In a single experiment on a man, the intravenous injection of 10 c.cm. reduced the clotting time of the blood by 40 per cent. Pecten has no action when added to shed blood. Further investigations are desirable, as purified pecten does not contain protein and is therefore not liable to produce "protein-shock."

"PEPTONE"

The use of crude "peptone" in the amelioration of hæmophilia has already been mentioned (on p. 147). Wallich and collaborators (1920) advise the subcutaneous injection of 5 c.cm. of a 5 per cent. solution, followed on the next day by an injection of 20 c.cm. Some "shock" is produced.

PITUITARY EXTRACTS

Extracts of the posterior lobe of the pituitary gland have been used in the arrest of hæmorrhage, particularly from the female genital organs. Pituitrin was used by Salinger (1918) as a prophylactic against hæmorrhage after tonsillectomy, 0·5 c.cm. being given to children and 1 c.cm. to adults, fifteen minutes before the operation. It is stated that in 62 cases out of 87 the post-operative bleeding was very slight, whilst in only two cases there was no evidence of prophylaxis The same writer used pituitrin in the arrest of secondary hæmorrhages and records success in thirteen cases. Stoudsinsky (1911) stated, however, that pituitary extracts yield inconstant results. This may be due to contamination with the anti-coagulant found in the anterior lobe of the gland.

A considerable amount of attention has been given to the physiological action of the extract of the posterior lobe of the pituitary gland. Emile-Weil and Boyé (1909, 1, 2) demonstrated that its introduction into the circulation produces hypercoagulability of the blood. Fiessly (1922) regards this reaction as similar to that of small amounts of "peptone" on circulating

plasma, and thus classes the extract with those substances which disturb the colloidal complexes of the blood. From clinical observations, Perrin and his collaborators (1922) conclude that the changes in blood pressure and leucopenia produced by the extract are, in some way, correlated with production of hypercoagulability. They also suggest direct action on plasma complexes. La Barre (1924) found that the substances present in the posterior lobe are incapable of provoking blood coagulation, but they accelerate the normal mechanism of thrombin-formation and also slightly accelerate the action of thrombin on fibrinogen. In addition to these augmentor actions on blood coagulation, the extract primarily constricts the arterioles and thus raises the blood pressure, the vasoconstrictor action being less marked and more prolonged than that of adrenaline. Vasodilatation may follow, but is of temporary duration. Owing to the dual action on blood plasma and on the arterioles, the use of pituitary extract appears to be advisable when superficial operations are performed on individuals with a predisposition to prolonged bleeding. Pituitary extracts bring both the gravid and non-gravid uterus into powerful contraction, and it is this quality which led to their use in obstetric practice.

A controversy exists as to whether the posterior lobe of the pituitary gland contains one or more active principles. From the chemical side Abel (1924) maintains that there is a single principle, but the observations of Dale and Dudley (1926) point to the presence of at least three active substances. By fractionation with butyl alcohol, they separated a vasopressor substance from a body which is a uterine stimulant. A third fraction was found which modifies renal diuresis. More recently Messrs. Parke, Davis & Co. have almost completely separated the vasopressor and oxytocic fractions. Experiments are in progress in my laboratory by Dr. F. R. Curtis and myself on the action of these principles on blood plasma. Preliminary results indicate that the intravascular injection of the pressor fraction produces hypercoagulability of the blood, whilst the oxytocic fraction retards blood clotting. The previous intravenous injection of heparin suppresses the augmentor action of the pressor fraction on blood clotting, but does not prevent the initial rise of blood pressure.

PROTOGULIN

This hæmostatic is a preparation of thrombin made and standardised in the Physiological Laboratory of St. Mary's Hospital, London. It is the most active of the hæmostatics mentioned in Table IV. (p. 153). It is recommended by the vendors in epistaxis and in external hæmorrhages arising from any cause. Colyer (1923) advises its use in dental surgery.

SERUMS

Serial subcutaneous injections of 10 to 20 c.cm of anti-diphtheritic serum were first used by Emile-Weil (1908) in the treatment of hæmophilia and have passed into common practice in Germany (Morawitz, 1926, 1). Marked temporary improvement, often accompaned with the cessation of bleeding from wounds, frequently follows the use of serial injections of normal horse serum (Vines, 1920; Mills, 1926, 1). Vines employs 10 c.cm. as a sensitising dose, followed by 2 minims ten days later, to produce an anaphylactic reaction. The same writer records a similar improvement after a single subcutaneous injection of 3 c.cm. of normal sheep serum, but this was accompanied by urticaria and a slight rise of temperature. (A like reaction may occur after serial injections of horse serum)

Intradermal injections of rabbit's serum, which has been sensitised by horse serum (the serum anthéma), have been used in the control of hæmoptysis, of intestinal bleeding during typhoid fever, and that of hæmophilic states (Dufour and Le Hello, 1921). A considerable amount of independent testimony to the value of the treatment is given in this paper. Anti-streptococcic serum is used by Yearsley (1925) in the treatment of hæmorrhages of the throat arising from infection by streptococci. In one case a single injection of 20 c.cm. sufficed; in another, 20 c.cm. almost arrested bleeding, whilst a second injection on the next day completely arrested oozing. Wilcox (1923–4) points out that the beneficial action of horse serum in arresting some hæmorrhages may be due to its anti-streptococcic activity. A combination of injections of heterologous serum ("artfreundes serum") and treatment with calcium preparations (either calcium lactate or Merck's kalzan) has been used in purpura hæmorrhagica (Morawitz, 1926, 1).

Horse serum has been used orally in the treatment of hæmorrhage, but the results appear inconstant (*cf.* Hurd, 1915; Graham, 1918; Hill, 1918). Its value should again be tested, the administration being followed by the giving of a glass of iced water, as in the use of fibrogen.

THROMBOPLASTINS (of Armour, Squibb and Leddele)

These preparations are extracts of brain tissue and owe their activity mainly to the presence of cephalin. Armour's product is standardised by its coagulant action on oxalated plasma, which does not give an adequate indication of its activity. Its local use is recommended by the makers in post-operative hæmorrhages, particularly in bleeding from bones, the nose and throat. Injections into the site of bleeding are suggested in dental cases when

local application fails. Hanzlik and Weidenthal (1919–1920) reported favourably on its value. Thromboplastin (Squibb) is prepared from ox brain by the method of Hess (1916). Separate preparations are supplied for local and hypodermic use, the latter to be employed in local injections of 2–3 c.cm. in dental hæmorrhage and in subcutaneous injections up to 40 c.cm. for post-operative hæmorrhage and in hæmophilia.

THYMUS NUCLEIC ACID

It has been shown that the intravenous injection of small amounts of neutralised thymus nucleic acid produces hypercoagulability of the blood (Pickering and Hewitt, 1924, 1), and it has been suggested that the value of injections of this substance should be tested in hæmophilia (Christie and Gulland, 1927). If this is attempted great care should be exercised, as moderate overdosage produces both hypocoagulability and deplateletisation of the blood.

SODIUM CITRATE

R. Weil (1916–1917) observed hypercoagulability of blood shed after intravenous injections of small amounts of this salt. Ottenberg (1916) noted a temporary increase of the coagulability of the blood of a hæmophilic after the administration of 0·6 gram of sodium citrate, followed by a decrease of coagulability. Kinsella and Broun (1920) reported the control of hæmorrhage of the lung in five patients by the intravenous injection of sodium citrate. Some shock occurred. Neuhoff and Hirschfeld (1922) administered sodium citrate to 500 individuals, using both intravenous and intramuscular injections. As much as 15 c.cm. of a 30 per cent. solution was injected into the buttocks of adults, made up with novocaine to allay the pain arising from the injection. In normal individuals a pronounced shortening of the clotting time of the blood rapidly follows the injection. It lasts 2 or 3 hours, and there is a gradual return to a normal speed of clotting in 24 or 48 hours. These reactions are utilised in prophylaxis against post-operative bleeding. Treatment by injections of sodium citrate has also been used with success in hæmatemesis, hæmoptysis, in intracranial and abdominal hæmorrhages, and in those arising from trauma of the thorax and liver. The value of sodium citrate as an internal hæmostatic is confirmed by a number of observers (Chienisse, 1922; Higgins and Fisher, 1924; Renaud and Juge, 1924). Of these investigators, Chienisse (1922) describes the arrest of excessive hæmorrhage in three cases of carcinoma, whilst Renaud and Juge (1924), using 10 to 25 c.cm. of a 30 per cent. solution, arrested severe bleeding in 14 of 17 cases of carcinoma. Other methods of

administration are less successful. The effects produced by ingestion are slow and variable, whilst subcutaneous injection causes considerable pain and local œdema (Chienisse, 1922). The intravenous injection of 20–30 c.cm. of a solution made by mixing 20 grams of sodium citrate with 10 grams of M_gSO_4 and 100 grams of water is recommended by Dax (1927) for the treatment of hæmorrhages following prostatectomy. It is claimed that this mixture does not produce the hypertensive action which follows the use of sodium citrate alone. The solution should be used immediately, as it becomes toxic on keeping.

APPENDIX B

GENERAL BIBLIOGRAPHY

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APPENDIX C

BIBLIOGRAPHY OF METHODS OF ESTIMATING FIBRINOGEN AND FIBRIN

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